ROBUST SUMMARIES OF STUDIES USED TO CHARACTERIZE THE REFINERY GASES CATEGORY

Submitted to the USEPA

by

The American Petroleum Institute (API)

Petroleum HPV Testing Group

www.petroleumhpv.org

Consortium Registration # 1100997

June 10, 2009

ROBUST SUMMARIES OF STUDIES USED TO CHARACTERIZE THE REFINERY GASES CATEGORY

Table of Contents

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

71-43-2	Benzene
74-93-1	Methanethiol
75-08-1	Ethanethiol
75-28-5	Isobutane (for C1 – C4 hydrocarbon fraction)
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
630-08-0	Carbon monoxide
1333-74-0	Hydrogen
5188-07-8	Methanethiol, sodium salt (for hydrogen sulfide and mercaptans, only)
7664-41-7	Ammonia
7727-37-9	Nitrogen
7783-06-4	Hydrogen sulfide
No CASRN	light naphtha (gasoline) stream (for C5 – C6 hydrocarbon fraction)

Physico-Chemical Data

PHYSICAL-CHEMICAL SIDS **MELTING POINT**	
Category Chemical :	Refinery Gases, multiple CAS numbers
Test Substance :	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	<u>Major and minor constituents comprising the CAS numbers in the Refinery Gases Category:</u> Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons C5-C6 Hydrocarbons

Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type :	
RESULTS	
Melting Indicator :	
Melting Point Input type :	Value or Range?
Melting Point Range : Temperature:	
Results Remarks :	Melting point values for major and minor constituents in Refinery Gases cited in referenced literature sources. **Constituent** — **MP, °C** — **Reference** Hydrogen — -259 — Budavari (1996) Ammonia — -77.7 — Budavari (1996) Hydrogen sulfide — -85.5 — Budavari (1996) Methanethiol — -123 — O'Neil (2001) Ethanethiol — -148 — Lide and Milne (1994) Carbon monoxide — -205 — Budavari (1996) Carbon dioxide — -56.5 — Lide (1994) Nitrogen — -210 — Budavari (1996)

	Benzene	5.5	Budavari (1996)
	1,3 butadiene	-109	Budavari (1996)
	C1-C4 Hydrocarbons	-189.7 to -138.4	API (2001)
	C5-C6 Hydrocarbons	-130 to –95	EPA (2000)

STUDY/METHOD	
Key Study Sponsor Indicator :	
Year Study Performed :	
Method/Guideline Followed :	
Method/Guideline and Test Condition Remarks:	
GLP :	
Study Reference :	Budavari, S. (ed.). 1996. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck and Co., Inc., Whitehouse Station, NJ. 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. 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.

	Lide, D.R. (ed.). 1994 – 1995. CRC Handbook of Chemistry and Physics. 75th ed. CRC Press Inc., Boca Raton, Fl. p. 4-50. API (American Petroleum Institute). 2001. Petroleum Gases Test Plan. Submitted to the U.S. EPA by The Petroleum HPV Testing Group, American Petroleum Institute, Washington, DC. EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S. Environmental Protection Agency, Washington, DC.
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 refinery gases.

PHYS CHEM **BOILING POINT**	
Category Chemical:	Refinery Gases, multiple CAS numbers
Test Substance:	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Refinery Gases Category: Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons C5-C6 Hydrocarbons

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 Refinery Gases cited in referenced literature sources.

Constituent	BP, °C	Reference
Hydrogen	-252.8	Budavari (1996)
Ammonia	-33	Budavari (1996)
Hydrogen sulfide	-60.3	Budavari (1996)
Methanethiol	5.95	O'Neil (2001)
Ethanethiol	35.1	Lide and Milne (1994)
Carbon monoxide	-191.5	Budavari (1996)
Carbon dioxide	-78.5	Lide (1994)
Nitrogen	-196	Budavari (1996)

	Benzene	80.1	Budavari (1996)
	1,3 butadiene	-4.5	Budavari (1996)
	C1-C4 Hydrocarbons	-164 to -0.5	API (2001)
	C5-C6 Hydrocarbons	36 to 69	EPA (2000)

STUDY/METHOD	
Key Study Sponsor Indicator:	
Year Study Performed:	
Method/Guideline Followed:	
Method/Guideline and Test Condition Remarks:	
GLP:	
Study Reference:	Budavari, S. (ed.). 1996. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck and Co., Inc., Whitehouse Station, NJ. 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. 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.

	Lide, D.R. (ed.). 1994 – 1995. CRC Handbook of Chemistry and Physics. 75th ed. CRC Press Inc., Boca Raton, Fl. p. 4-50. API (American Petroleum Institute). 2001. Petroleum Gases Test Plan. Submitted to the U.S. EPA by The Petroleum HPV Testing Group, American Petroleum Institute, Washington, DC. EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S. Environmental Protection Agency, Washington, DC.
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 refinery gases.

PHYSICAL-CHEMICAL SIDS **VAPOR PRESSURE**	
Category Chemical:	Refinery Gases, multiple CAS numbers
Test Substance:	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Refinery Gases Category: Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons C5-C6 Hydrocarbons

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 Refinery Gases cited in referenced literature sources.

Constituent	VP, hPa	Reference
Hydrogen	1,653,198	Ohe (1976)
Ammonia	10,013	Daubert and Danner (1989)
Hydrogen sulfide	20,798	Daubert and Danner (1989)
Methanethiol	20,665	Daubert and Danner (1989)
Ethanethiol	529	Daubert and Danner (1989)
Carbon monoxide	20,664,972	EPA (2000)
Carbon dioxide	64,395	Daubert and Danner (1989)
Nitrogen	1013[1]	Weast (1984)
Benzene	126	Daubert and Danner (1989)

	1,3 butadiene 2,813 Daubert and Danner (1989) C1-C4 Hydrocarbons 3,796 to 350,000 API (2001) C5-C6 Hydrocarbons 201 to 685 EPA (2000) [1] VP value for nitrogen is for -196 °C. All other values at 25 °C.
STUDY/METHOD	
Key Study Sponsor Indicator:	
Year Study Performed:	
Method/Guideline Followed:	
Method/Guideline and Test Condition Remarks:	
GLP:	
Study Reference:	Ohe S. 1976. Computer Aided Data Book of Vapor Pressure. Data Book Publ. Co, Tokyo, Japan. Daubert, T.E., and R.P. Danner. 1989. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Taylor and Francis, Washington, D.C. Weast, R.C. (ed.). 1984 – 1985. Handbook of Chemistry and Physics. 65th ed. CRC Press, Inc. Boca Raton, FL., p. D-219.

	EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S. Environmental Protection Agency, Washington, DC. API (American Petroleum Institute). 2001. Petroleum Gases Test Plan. Submitted to the U.S. EPA by The Petroleum HPV Testing Group, American Petroleum Institute, Washington, DC.
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 refinery gases.

PHYSICAL-CHEMICAL SIDS **PARTITION COEFFICIENT**	
Category Chemical:	Refinery Gases, multiple CAS numbers
Test Substance:	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Refinery Gases Category: Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons C5-C6 Hydrocarbons

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 Refinery Gases cited in referenced literature sources.

Constituent	Log Kow	Reference
Hydrogen	N/A	
Ammonia	-1.14	BASF AG (1992)
Hydrogen sulfide	0.45	BASF AG (1992)
Methanethiol	0.65	Abraham et al. (1994)
Ethanethiol	1.27	EPA (2000)
Carbon monoxide	1.78	EPA (2000)
Carbon dioxide	0.83	EPA (2000)
Nitrogen	0.67	Hansch et al. (1995)
Benzene	2.13	Hansch et al. (1995)
1,3-butadiene	1.99	Hansch et al. (1995)

	C1-C4 Hydrocarbons	1.09 – 2.8	API (2001)
	C5-C6 Hydrocarbons	3.4 – 3.9	EPA (2000)
STUDY/METHOD			
Key Study Sponsor Indicator:			
Year Study Performed:			
Method/Guideline Followed:			
Method/Guideline and Test Condition Remarks:			
GLP:			
Study Reference:	BASF AG, Abteilung Analytik; unveroeefentlichte Untersuchung (BRU 92.004). [cited in ECB (European Chemicals Bureau). 2000. IUCLID Dataset for Ammonia, CAS No. 7664-41-7. European Commission, Brussels, Belgium] BASF AG, Abteilung Analytik; unveroeffentlichte Untersuchung (BRU 92.003). [cited in ECB (European Chemicals Bureau). 2000. IUCLID Dataset for Hydrogen Sulphide, CAS No. 7783-06-4. European Commission, Brussels, Belgium] Abraham M.H., H.S. Chadha, G.S. Whiting, and R.C. Mitchell. 1994. Hydrogen Bonding. 32. An analysis of water-octanol and water-alkane partitioning and the Δ-log P parameter of Seiler. J. Pharm. Sci. 83(8):1085-100. 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. API (American Petroleum Institute). 2001. Petroleum Gases Test Plan. Submitted to the U.S. EPA by		

	The Petroleum HPV Testing Group, American Petroleum Institute, Washington, DC. Hansch, C., A. Leo, and D. Hoekman. 1995. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. American Chemical Society, Washington, DC, p. 3.
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 refinery gases.

HIGH PRODUCTION VOLUME INFORMATION SYSTEM (HPVIS)

PHYS CHEM **WATER SOLUBILITY**	
Category Chemical :	Refinery Gases, multiple CAS numbers
Test Substance :	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Refinery Gases Category: Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons

	C5-C6 Hydrocarbons
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 Refinery Gases cited in referenced literature sources. **Water Solubility mg/L** / **Reference** Hydrogen 1.62 Venable and Fuwa (1922) Ammonia 340,000 Budavari (1996) Hydrogen sulfide 3,980 Kirk-Othmer (1991) Methanethiol 15,400 Hine and Mookerjee (1975) Ethanethiol 15,600 Wakita et al. (1986) Carbon monoxide 24,582 EPA (2000) Carbon dioxide 1,480 EPA (2000) Nitrogen 18,100 EPA (2000) Benzene 1,790 May et al. (1983) 1,3-butadiene 735 McAuliffe (1966) C1-C4 Hydrocarbons 24 – 61 API (2001) C5-C6 Hydrocarbons 9.5 – 38 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. Budavari, S. (ed.). 1996. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck and Co., Inc., Whitehouse Station, NJ. p. 87. Kirk-Othmer Encyclopedia of Chemical Technology. 4th ed. Volumes 1, 1991-Present. John Wiley and Sons, New York, NY. p. V23 277. Hine J. and P.K. Mookerjee. 1975. The intrinsic hydrophilic character of organic compounds. Correlations in terms of structural contributions. J. Org. Chem. 40: 292-8. Wakita, K., M. Yoshimoto, S. Miyamoto, and H. Watanabe. 1986. A method for calculation of the aqueous solubility of organic compounds by using new fragment solubility constants. Chem. Pharm. Bull. 34(11):4663-4681. May, W.E., D.P. Wasik, M.M. Miller, Y.B. Tewari, J.M. Brown-Thima, and R.N. Goldberg. 1983. Solution Thermodynamics of some slightly soluble hydrocarbons in water. J. Chem. Ref. Data 28: 197-0200. [Cited in: NLM (U.S. National Library of Medicine). 2005. Benzene, Hazardous Substance Data Bank Number 35. Available through the TOXNET Toxicology Data Network, http://toxnet.nlm.nih.gov] McAuliffe, C. 1966. Solubility in water of paraffin, cycloparaffin, olefin, acetylene, cycloolefin and aromatic hydrocarbon. J. Phys. Chem. 70: 1267-1275. API (American Petroleum Institute). 2001. Petroleum Gases Test Plan. Submitted to the U.S. EPA by The Petroleum HPV Testing Group, American Petroleum Institute, Washington, DC. EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S. Environmental Protection Agency, Washington, DC.
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 refinery gases.

ENVIRONMENTAL FATE AND PATHWAYS

FATE SIDS **PHOTODEGRADATION**	
Category Chemical :	Refinery Gases, multiple CAS numbers
Test Substance :	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	<u>Major and minor constituents comprising the CAS numbers in the Refinery Gases Category:</u> Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons

	C5-C6 Hydrocarbons
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 refinery 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**
Hydrogen	N/A	Non-reactive in troposphere (Boikess and Edelson, 1978)
Ammonia	16	For an OH^- concentration of $3x10^6$ molecules/cm^3 (Perry et al., 1976)
Hydrogen sulfide	3.3	ECB (2000)
Methanethiol	0.5	For an OH^- concentration of $5x10^5$ molecules/cm^3 (Atkinson, 1989)
Ethanethiol	0.4	For an OH^- concentration of $5x10^5$ molecules/cm^3 (Atkinson, 1989)
Carbon monoxide	1559	AOPWIN model in EPI-Suite™ EPA (2000)
Carbon dioxide	N/A	Non-reactive in troposphere (Boikess and Edelson, 1978)
Nitrogen	N/A	Non-reactive in troposphere (Sawyer and McCarty, 1978)
Benzene	5.5	AOPWIN model in EPI-Suite™ EPA (2000)
1,3-butadiene	0.16	AOPWIN model in EPI-Suite™ EPA (2000)
C1-C4 Hydrocarbons	0.16 to 960	Values for butane and methane, respectively. AOPWIN model

			in EPI-Suite™ EPA (2000)
	C5-C6 Hydrocarbons	2 to 2.5	Values for pentane and hexane, respectively. AOPWIN model in EPI-Suite™ EPA (2000)
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. Perry, R.A., A. Atkinson, and J.N. Pitts, Jr. 1976. Rate Constants for the Reactions OH + $H_2S \rightarrow$ SH and OH + $NH_3 \rightarrow H_2O + NH_2$ over the Temperature Range 297 – 427°K. J. Chem. Phys. 64:3237-3239. [as cited in NRC, 1979] European Chemicals Bureau. IUCLID Dataset for Hydrogen Sulphide (CAS 7783-06-4). Brussels, Belguim. Atkinson, R. 1989. J. Phys. Chem. Ref. Data. Monograph No. 1 [as cited in NLM (U.S. National Library of Medicine. 2005. Hazardous Substance Data Bank. Available through TOXNET Toxicology Data		

	Network, http://toxnet.nlm.nih.gov] 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 refinery gases.

HIGH PRODUCTION VOLUME INFORMATION SYSTEM (HPVIS)

FATE SIDS **STABILITY IN WATER**	
Category Chemical :	Refinery Gases, multiple CAS numbers
Test Substance :	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and **Other Test Substance Comments**:	<u>Major and minor constituents comprising the CAS numbers in the Refinery Gases Category:</u> Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons C5-C6 Hydrocarbons

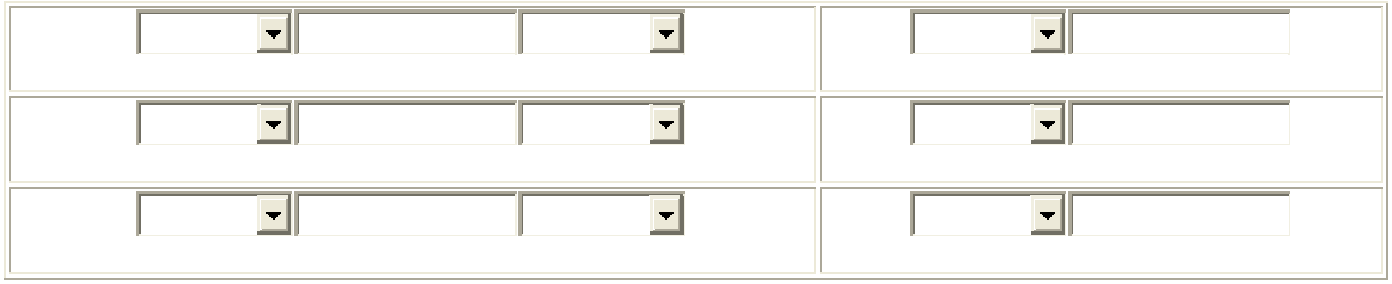
Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type :	

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 Refinery 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 Refinery 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 refinery gases.

FATE SIDS TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS (FUGACITY)	
Category Chemical :	Refinery Gases, multiple CAS numbers
Test Substance :	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Refinery Gases Category: Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons C5-C6 Hydrocarbons

Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type :	
Results	
Fugacity/Distribution Result Description:	Multimedia (Fugacity) Modeling

Test Results :

Refinery Gas — **PERCENT DISTRIBUTION** — **Suspended Sediment**

Constituent	Air	Water	Soil	Sediment	Suspended Sediment	Biota
hydrogen sulfide	100	<0.1	<0.1	<0.1	<0.1	<0.1
methanethiol	99.2	0.77	<0.1	<0.1	<0.1	<0.1
ethanethiol	98.2	1.7	<0.1	<0.1	<0.1	<0.1
ammonia	88.0	11.9	<0.1	<0.1	<0.1	<0.1
1,3-butadiene	100	<0.1	<0.1	<0.1	<0.1	<0.1
benzene	99.0	0.88	<0.1	<0.1	<0.1	<0.1
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 :	
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 Refinery 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 refinery gases.

FATE SIDS BIODEGRADATION	
Category Chemical :	Refinery Gases, multiple CAS numbers
Test Substance :	Refinery Gases, various CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Refinery Gases Category: Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons C5-C6 Hydrocarbons

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 Refinery 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. In contrast, ammonia can be readily oxidized to nitrite under aerobic

	conditions by autotrophic nitrifying bacteria (Sawyer and McCarty, 1978). Carbon monoxide has been reported to be microbially oxidized to CO_2 in pure cultures by a number of microbial species. It was also shown to be rapidly converted to CO_2 by indigenous soil microbial communities (Bartholomew and Alexander, 1979). Methanethiol can be both evolved and consumed in nature. It is produced by a variety of organisms through the decay of sulfur-containing organic matter under anoxic conditions (Kiene and Capone, 1988). Methanethiol is known to undergo both aerobic and anaerobic biodegradation, but Lomans et al. (1999) reported methanogenesis was the major mechanism of methanethiol consumption under an anoxic environment. Visscher and Taylor (1993) showed that a pure culture of *Thiobacillus* sp. grown in the presence of dimethyl sulfide oxidized a range of alkylthiols including methanethiol and ethanethiol. Hydrogen sulfide does not biodegrade *per se*, but bacteria play an important role in the cycling of sulfur in the environment. The reduction of sulfate to hydrogen sulfide occurs in anoxic environments by anaerobic bacteria, and this can cause serious odor problems when sufficient amounts of sulfide are produced (Sawyer and McCarty, 1978). Conversely, hydrogen sulfide can be oxidized to elemental sulfur and sulfate by a number of bacteria (EPA, 1986). Much of this cycling of sulfur occurs in sediments at the boundary layer between oxic and anoxic conditions (EPA, 1986). Biodegradation of the hydrocarbon components in refinery 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 :	Sawyer, C.N., and P.L. McCarty. 1978. Chemistry for Environmental Engineering, 3rd edition. McGraw-Hill Book Company, New York, New York. 532 p. Bartholomew, G.W., and M. Alexander. 1979. Microbial Metabolism of Carbon Monoxide in Culture and In Soil. App. Environ. Microbiol. 37(5):932-937. Kiene, R.P., and D.G. Capone. 1988. Microbial Transformations in Methylated Sulfur Compounds in Anoxic Salt Marsh Sediments. Microb. Ecol. 15: 275-91. Lomans, B.P., H.J.M. Op den Camp, A. Pol, and G. D. Vogels. 1999. Anaerobic Versus Aerobic Degradation of Dimethyl Sulfide and Methanethiol in Anoxic Freshwater Sediments. App. Environ. Microbiol. 65(2):438-443. Visscher, P.T., and B.F. Taylor. 1993. Aerobic and Anaerobic Degradation of a Range of Alkyl Sulfides by a Denitrifying Marine Baceterium. App. Environ. Microbiol. 59(12):4083-4089. EPA (United States Environmental Protection Agency). 1986. Health and Environmental Effects Profile for Hydrogen Sulfide. Report No. ECAO-CIN-026A. [Cited in: NLM (U.S. National Library of Medicine). 2005. Hazardous Substance Data Bank. Available through the TOXNET Toxicology Data Network, http://toxnet.nlm.nih.gov]

	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 refinery gases.

ECOTOXICITY

Acute Toxicity to Aquatic Vertebrates	
Test Substance	
Category Name: Refinery Gases Category	
Category Chemical :	Refinery Gases, multiple CAS numbers
Test Substance :	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments :	Major and minor constituents comprising the CAS numbers in the Refinery Gases Category: Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene

	C1-C4 Hydrocarbons C5-C6 Hydrocarbons
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:	Refinery 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 refinery gases. Some constituents, namely N_2, H_2, CO_2, and CO, 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. However, other constituent substances in refinery gases have well documented aquatic hazards and were considered the potential toxic drivers in these streams. These included ammonia, hydrogen sulfide,

	methanethiol, ethanethiol, benzene, and hydrocarbons. Available aquatic toxicity data for these substances are tabulated and presented below. **Refinery Gas Range of Toxicity Values** **Constituent (LC50, mg/L) Reference** Ammonia 0.083 – 4.6 EPA, 1986 Hydrogen sulfide 0.007 – 0.2 Fung and Bewick (1980); Smith et al. (1976) Methanethiol 0.5 ≤ LC50 ≤ 1.75 Haydu et al. (1952) Ethanethiol no data Benzene 5.3 – 35.7 DeGraeve et al. (1982); Brooke (1987) C1-C4 Hydrocarbons 11.3 – 167 API (2001) C5-C6 Hydrocarbons 3.9 – 9.5 EPA (2000)
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 refinery gases.
Key Study Sponsor Indicator:	
Reference	
Reference:	US EPA (United States Environmental Protection Agency). 1986. Quality Criteria for Water: 1986. EPA 440/5-86-001, U.S. EPA, Office of Water,

Washington, DC.

Fung, D.K., and P.H. Bewick. 1980. Short-Term Toxicity of Aqueous Hydrogen Sulfide to Representative Fish Species of Lake Huron. In: Eaton, J.G., P.R. Parrish, and A.C. Hendricks (Eds.), Aquatic Toxicology and Hazard Assessment, 3rd Symposium, ASTM STP 707, Philadephia, PA.

Smith, L.L. Jr., D.M. Oseid, and L.E. Olson. 1976. Acute and chronic toxicity of hydrogen sulfide to the fathead minnow, *Pimephales promelas*. Environ. Sci. Technol. 10(6):565-568.

Haydu, E.P., et al. 1952. The effect of kraft mill waste components on certain salmonoid fishes of the Pacific Northwest. TAPPI. 35(12):545-549.

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: Refinery Gases Category	
Category Chemical:	Refinery Gases, multiple CAS numbers
Test Substance:	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Refinery Gases Category: Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen

	Benzene 1,3-butadiene C1-C4 Hydrocarbons C5-C6 Hydrocarbons
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:

Refinery 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 refinery gases. Some constituents, namely N_2, H_2, CO_2, and CO, 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.

However, other constituent substances in refinery gases have well documented aquatic hazards and were considered the potential toxic drivers in these streams. These included ammonia, hydrogen sulfide, methanethiol, ethanethiol, benzene,

and hydrocarbons. Available aquatic toxicity data for these substances are tabulated and presented below.

Refinery Gas Consituent	Range of Toxicity Values (EC50, mg/L)	Reference
Ammonia	0.53 – 22.8	EPA (1986)
Hydrogen Sulfide	0.022 – 1.07	Smith et al. (1975); Oseid and Smith (1974)
Methanethiol	1.32 ≤ EC50 ≤ 2.46	Mercaptans/Thiols Council (2001)
Ethanethiol	90 – 280	Maas (1990)
Benzene	59.6 – 682	MacLean et al. (1989); Eastmond et al. (1984)
C1-C4 Hydrocarbons	12.7 – 164	API (2001)
C5-C6 Hydrocarbons	4.6 – 10.7	EPA (2000)

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 refinery gases.
Key Study Sponsor Indicator:	

Reference

Reference:	US EPA (United States Environmental Protection Agency). 1986. Quality Criteria for Water: 1986. EPA 440/5-86-001, U.S. EPA, Office of Water, Washington, DC. Smith, L.L. Jr. and D.M. Oseid. 1975. Chronic Effects of Low Levels of Hydrogen Sulfide on Freshwater Fish. Prog. Water Technol. 7(3/4):599-605. Oseid, D.M. and L.L. Smith, Jr. 1974. Factors Influencing Acute Toxicity Estimates of Hydrogen Sulfide to Freshwater Invertebrates. Water Research 8:739-746. Marcaptans/Thiols Council. 2001. Methyle mercaptans/methyl mercaptide High Production Volume Challenge Program Test Plan. Mercaptans/Thiols Council, Leesburg, VA Maas, J.L. 1990. Toxicity research with thiourea. Laboratory for Ecotoxicology. Institute for Inland Water Management and Waste Water Treatment, Report No. AOCE: 4p (DUT) 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:	Refinery Gases Category
Category Chemical :	Refinery Gases, multiple CAS numbers
Test Substance :	Refinery Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments :	Major and minor constituents comprising the CAS numbers in the Refinery Gases Category: Hydrogen Ammonia Hydrogen sulfide Methanethiol Ethanethiol Carbon monoxide Carbon dioxide Nitrogen Benzene 1,3-butadiene C1-C4 Hydrocarbons

	C5-C6 Hydrocarbons
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	**Exposure**	Type	**%**:	**Value**	**Mean Value or**	**Upper Mean**	**Units**:	**Basis for**	**Basis for**

Duration:	Units:			Description:	Lower Mean Value:	Value:		Effect:	Concentration:

Results Remarks:	Refinery 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 refinery gases. Some constituents, namely N_2, H_2, CO_2, and CO, 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. However, other constituent substances in refinery gases have well documented aquatic hazards and were considered the potential toxic drivers in these streams. These included ammonia, hydrogen sulfide, methanethiol, ethanethiol, benzene, and hydrocarbons. Available aquatic toxicity data for these substances are tabulated and presented below.

	Refinery Gas Range of Toxicity Constituent Values (EC50, mg/L) Reference Ammonia no data Hydrogen Sulfide no data Methanethiol no data Ethanethiol no data Benzene 29 Galassi et al. (1988) C1-C4 Hydrocarbons 1.3 – 95.7 API (2001) C5-C6 Hydrocarbons 3.1 – 7.0 EPA (2000)
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 refinery 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.

High Production Volume Information System (HPVIS)

Acute Toxicity

Test Substance

Category Chemical (CAS #):	74-93-1
Test Substance (CAS #):	Methanethiol (Methyl mercaptan)
Test Substance Purity/Composition and Other Test Substance Comments :	"as prescribed by 1.1 - 1.4" (Sections 1.1-1.4 were not included in the robust summaries)
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:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	10
Concentration:	
Dose:	0, 400, 600, 650, 680, 690, 700, 700, 800 ppm
Year Study Performed :	1981
Method/Guideline Followed:	No Data
GLP:	No Data
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 90 Exposure time: 4 hours Method: other: equivalent to OECD Guideline 403 Each dose group consisted of 5 male and 5 female rats, which were combined for a 4-h exposure or sham exposure to air in a customized 75-l glass chamber and then separated for observation over the subsequent 14-day period. Animals from any group that died during the 14-day period were examined for gross pathology, such as general or local hemorrhage and adhesions, and the survivors were sacrificed and examined as

	well. Mortality and such visually apparent behaviour as exploring, huddling, preening, and obvious distress were noted during the courses of the 4-hour exposures and sham exposures. The rats were deprived of food and water during actual exposure or sham exposure. LC50 values and 95% confidence limits were estimated by the classical method of Litchfield and Wilcoxon (1949).

TEST RESULTS

Concentration (LC/LD)

LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
	50	=	675		ppm (air)
	50	=	643	709	ppm (air)

Number of Deaths (Male):	
Number of Deaths (Female):	
Number of Deaths (Total):	45
Results Remarks:	The table summarizes the 14-d, 4-h LC50 determinations for methyl mercaptan. In all cases, any animal that survived the first 24 h after exposure survived to the end of the 2-wk observational period. There was no evidence of external bleeding from any orifice in rats that succumbed or survived. Dose-Response LC50 = 675 (643-709) Value: =675 ppm Mortality by dose: sham, 0/10 400, 0/10 600, 2/10 650, 5/10 680, 4/10

	690, 4/10 700, 10/10 700, 10/10 800, 10/10 Animals which survived the first 24 hours after exposure survived to the end of the 14 day observation period.
Conclusion:	LC50 = 675 (643-709) ppm
RELIABILITY/DATA QUALITY	
Reliability:	Valid with Restrictions (KS=2)
Reliability Remarks:	Non-guideline study; sufficient level of detail
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Tansy MF, Kendall FM, Fantasia J, Landin WE and Oberly R 1981. Acute and subchronic toxicity studies of rats exposed to vapors of methyl mercaptan and other reduced-sulfur compounds. J Toxicol Environ Health, 8, 71-88.

High Production Volume Information System (HPVIS)

Acute Toxicity

Test Substance

Category Chemical (CAS #):	75-08-1
Test Substance (CAS #):	Ethanethiol (Ethyl mercaptan)
Test Substance Purity/Composition and Other Test Substance Comments :	Gas chromatographic analysis showed it to be essentially the pure compound. Samples were subjected to infrared analysis and spectrograms were retained for future reference.
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 and mice
Other Species:	Rabbits (no strain indicated, not studied by inhalation
Mammalian Strain:	No data
Other Strain:	
Gender:	Males only
Number of Animals per Dose:	5 rats/ dose level 10 mice /dose level
Concentration:	

Dose:	Rats: 2600, 3150, 3573, 4438, 4832, 4868, 5100, 5125 ppm Mice: 2600, 3150, 3573, 4438, 4832 ppm
Year Study Performed :	1958
Method/Guideline Followed:	No Data
GLP:	No Data
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 42 (rats); 50 (mice) Exposure time: 4 hours Method: LC50 Each dose group consisted of 5 male rats or 10 male mice, which were exposed for 4 hr in a customized chamber and then separated for observation over the subsequent 14-day period. Most animals were held for observation for one month prior to being sacrificed. Tissue speciments were submitted for pathologic examination after having first been examined grossly and preserved. LC50 values were calculated by the method of Miller and Taintcr (1944). Note: eight other thiols were also tested by inhalation in this study.

TEST RESULTS

Concentration (LC/LD)

LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
	50 (rat)	=	4420		ppm (air)
	50 (rat)	=	4290	4541	ppm (air)
	50 (mouse)	=	2770		ppm (air)
	50 (mouse)	=	2661	2879	ppm (air)

Number of Deaths (Male):	14 (rats) 41 (mice)
Number of Deaths (Female):	Not applicable

Number of Deaths (Total):	14 (rats) 41 (mice)
Results Remarks:	The tables summarizes the 14-d, 4-h LC50 determinations for ethyl mercaptan. RATS: LC50 = 4420 (4290-4541) ppm Value = 4420 ppm Mortality by dose in ppm (14 days after exposure): 2600, 0/5 3150, 0/5 3573, 0/5 4438, 1/5 4832, 4/6 4868, 2/5 5100, 5/5 (all dead at 24 hr) 5125, 2/6 MICE: LC50 = 2770 (2661-2879) ppm Value = 2770 ppm Mortality by dose in ppm (14 days after exposure): 2600, 4/10 3150, 7/10 3573, 10/10 (all dead at 24 hr) 4438, 10/10 (all died between 0-4 hr) 4832, 10/10 (all died between 0-4 hr) Mice were more susceptible to the toxic effects of all thiols tests. Both rats and mice exhibited signs of intoxication. Maximum sublethal concentrations of all the thiols (including ethanethiol) induced characteristic symptoms of toxicity, i.e., increased respiration and restlessness, uncoordinated movement and staggering gait, muscular weakness, partial skeletal muscle paralysis beginning in hind limbs, light to severe cyanosis, tolerance of prone position, and mild to heavy sedation. Fatal responses usually followed one of two patterns: (1) animals exposed to maximal

	lethal concentrations died from respiratory arrest while in or shortly after removal from the chamber, and (2) those animals exposed to minimal lethal concentrations died while in a semiconscious condition of long duration. Animals exposed to ethanethiol very often remained in a semi-conscious condition of sedation and lethargy 4 to 6 hours post-exposure before showing signs of recovery. Most of the thiols were irritating to the mucous membranes within approximately 15 minutes after exposure of animals to high concentrations as evidenced by their rubbing of the eyes and nose, eye closure, occasional sneezing, watering or the eyes and and retracting of the head. Animals dying several hours after exposure to high vapor concentrations showed mild to severe hyperemia of the trachea and lungs. Moderate to near-lethal exposures to any given compound produced greater effects in mice than in rats. Characteristic findings, varying in degree of severity were as follows: liver changes consisting of cloudy swelling and fatty degeneration as early as 18 hours post-exposure, and necrosis which occasionally covered large areas, lung changes consisting of capillary engorgement, patchy edema and occasional hemorrhage; kidneys showed varying degrees of cloudy swelling, but more often mild to moderate;. The general signs of acute thiol poisoning exhibited by rats (as well as mice and rabbits) were uniform and were indicative of central nervous system depression and respiratory paralysis with death ensuing from respiratory failure.
Conclusion:	Rat:LC50 = 4420 (4290-4541) ppm Mouse: LC50 = 2770 (2661-2879) ppm
RELIABILITY/DATA QUALITY	
Reliability:	Valid with Restrictions (KS=2)
Reliability Remarks:	Nonguideline study; only one sex used.
Key Study Sponsor Indicator:	Key
REFERENCE	

Reference:	Fairchild, EJ and Stokinger, HE .1958.Toxicologic studies on organic sulfur compounds. I. Acute toxicity of some aliphatic and aromatic thiols (Mercaptans). Am. Ind. Hyg. Assoc. J. 19:171-189.

High Production Volume Information System (HPVIS)

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.

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:**

LC		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 #):	630-08-0
Test Substance (CAS #):	Carbon monoxide
Test Substance Purity/Composition and Other Test Substance Comments :	Chemically pure (CP) grade (Air Products and Cemicals, Inc., Allentown, PA)
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:	Rat
Other Species:	Mice and guinea pigs
Mammalian Strain:	Sprague-Dawley
Other Strain:	NIH/Nmri Swiss albino mice and FTD Hartley guinea pigs (results not reported here)

Gender:	Males
Number of Animals per Dose:	4-12, depending upon dose.
Concentration:	
Dose:	1600, 1800, 2000, 2200 ppm at normal atmospheric pressure (psig=0)
Year Study Performed :	1970
Method/Guideline Followed:	No Data
GLP:	No Data
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 4-12, depending upon dose Exposure time: 4 hours Method: Animals exposed for 4 hours to various concentrations of carbon monoxide under various pressures. Normal atmospheric pressure is 0 psig, although other atmospheric pressures were also evaluated in this experiment. Carbon monoxide concentrations were continuously monitored by an IR spectrophotometer or gas chromatograph. Immediately after the exposures, cardiac blood samples from dead animal. were collected and analyzed for carboxyhemoglobin concentration. The LC50 values and their 95% confidence limits were calculated by the method of Litchfield and Wilcoxon.

TEST RESULTS

Concentration (LC/LD)

LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
	50	=	1807		ppm (air)
	50	=	1598	1956	ppm (air)

Number of Deaths (Male):	

Number of Deaths (Female):	Not applicable
Number of Deaths (Total):	18
Results Remarks:	In general, all animals lost consciousness during the first 1-2 hr of exposure to CO. LC50: 1807 ppm (1598-1956 ppm) Mortality by dose: 1600 ppm, 1/4 1800 ppm, 2/4 2000 ppm, 4/8 2200 ppm, 11/12 Note: increasing atmospheric pressure did not significantly affect the LC50 values.
Conclusion:	LC50 = 1807 ppm (1598-1956 ppm)
RELIABILITY/DATA QUALITY	
Reliability:	Valid with Restrictions (KS=2)
Reliability Remarks:	Non guideline study . Single sex. Sufficient level of detail.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Rose CS, Jones RA, Jenkins LJ Jr, Siegel J.1970. The acute hyperbaric toxicity of carbon monoxide. Toxicol Appl Pharmacol *17*:752760.

Acute Toxicity

Test Substance	
Category Chemical (CAS #):	7664-41-7
Test Substance (CAS #):	Ammonia
Test Substance Purity/Composition and Other Test Substance Comments :	Matheson, East Rutherford, NJ
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:	No data
Species:	Mouse
Other Species:	
Mammalian Strain:	Albino ICR
Other Strain:	
Gender:	Male

Number of Animals per Dose:	12
Concentration:	
Dose:	Experiment 1 – 0, 1190, 3950, 4490 ppm Experiment 2 - 0, 1340, 2130, 4860 ppm Experiment 3 – 0, 3440, 4220, 4860 ppm
Year Study Performed :	1982
Method/Guideline Followed:	No Data
GLP:	No Data
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 12 per dose (3 experiments at four doses = 144 total) Exposure time: 1 hour Groups of 12 mice each were exposed simultaneously to three concentrations of NH_3 gas (1,190-4,860 ppm depending on which of the three experiments) in air using a dynamic inhalation exposure system. The control group was exposed to air only. The mice were allowed free access to food and water during the experiment, except while in chamber tubes. The mice were acclimated for at least 1week prior to the experiment. Sampling and analyses were conducted every 3-5 minutes. Animals were observed for 14 days following exposure.

TEST RESULTS

Concentration (LC/LD)					
.D	**%:**	**Value Description:**	**Value or Lower Concentration:**	**Upper Concentration:**	**Units:**
	50	=	4230		ppm (air)
			4070	4400	ppm

Number of Deaths (Male):	Experiment 1 – 11 Experiment 2 - 48 Experiment 3 – 15

Number of Deaths (Female):	Not applicable
Number of Deaths (Total):	Experiment 1 – 11 Experiment 2 - 48 Experiment 3 – 15
Results Remarks:	LC50: 4230 ppm 95% C.L. = 4070-4400 ppm At high NH_3 concentrations, mortality usually occurred within 30 minutes of initiation of exposure. Twenty-five percent of animals exposed to 3950 ppm did not survive. Percent mortality in higher concentrations (4220-4860 ppm) ranged from 41.6 to 100%. The lungs of animals that died during exposure were congested with evidence of hemorrhage. The lungs of animals from every treatment group sacrificed displayed a mild to moderate degree of chronic focal pneumonitis histologically. The liver and heart weights were increased in animals that died during exposure to 4860 ppm. Experiment 1 4490 ppm -66.6 % mortality 3950 - 25.0% 1190 - 0 0 - 0 Experiment 2 4860ppm -100 % mortality 2130 - 0 1340 - 0 0 - 0 Experiment 3 4860 ppm - 83.3% mortality 4220 - 41.6 3440 - 0 0 - 0 The LCSO and 95% confidence interval were determined by the method of Litchfield and Wilcoxon. Mean body weight changes were analyzed using the "t" test for paired

	observations while mean organ-to-body weight ratios were compared using either the Student "t" test or the one-way ANOVA followed by Duncan's Multiple Range Test.
Conclusion:	LC50 = 4230 ppm (1 hr)
RELIABILITY/DATA QUALITY	
Reliability:	Valid with Restrictions (KS=2)
Reliability Remarks:	Non standard time period for LD50 test, single sex used
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Kapeghian JC, Mincer HH, Jones AL, Verlangieri, AJ, and IW Waters. 1982. Acute inhalation toxicity of ammonia in mice. Bull Environ Contam Toxicol 29:371-378.

High Production Volume Information System (HPVIS)

Acute Toxicity

Test Substance	
Category Chemical (CAS #):	7783-06-4
Test Substance (CAS #):	Hydrogen sulfide

Test Substance Purity/Composition and Other Test Substance Comments :	Hydrogen sulfide supplied by Union Carbide Corp
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:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	10
Concentration:	
Dose:	sham, 400, 440, 475, 500, 525, 554, 600 ppm
Year Study Performed :	1981
Method/Guideline Followed:	No Data
GLP:	No Data

Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 5/sex Exposure time: 4 hours Method: Animals exposed for 4 hours to hydrogen sulfide or air and observed for 14 days and examined for gross pathology, such as general or local hemorrhage and adhesions. Mortality and visually apparent behavior such as exploring, huddling, preening, and obvious distress were noted during the 4 hr exposure. The rats were deprived of food and water during exposure.

TEST RESULTS

Concentration (LC/LD)

D	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
	50	=	444		ppm (air)
	50	=	416	473	ppm (air)

Number of Deaths (Male):	
Number of Deaths (Female):	
Number of Deaths (Total):	48
Results Remarks:	LC50: 444 (416-473 ppm) Mortality by dose: sham, 0/10 400, 3/10 440, 3/10 475, 7/10 500, 8/10 525, 8/10 554, 9/10 600, 10/10 Animals which survived the first 24 hours after exposure survived to the end of the 14

	day observation period.
Conclusion:	LC50 = 444 ppm (416-473 ppm)
RELIABILITY/DATA QUALITY	
Reliability:	Valid with Restrictions (KS=2)
Reliability Remarks:	Comparable to a guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Tansy MF, Kendall FM, Fantasia J, Landin WE and Oberly R 1981. Acute and subchronic toxicity studies of rats exposed to vapors of methyl mercaptan and other reduced-sulfur compounds. J Toxicol Environ Health, 8, 71-88.

High Production Volume Information System (HPVIS)

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.

High Production Volume Information System (HPVIS)

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.

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.

High Production Volume Information System (HPVIS)

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	74-93-1
Test Substance:	Methanethiol (Methyl mercaptan)
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:	Subchronic 90 day
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley
Other Strain:	Not applicable
Gender:	Both M/F
Number of Animals per Dose:	31
Concentration:	
Dose:	0, 2, 17 or 57 ppm
Year Study Performed:	1981
Method/Guideline Followed:	Other
GLP:	No data
Exposure Period:	90 Days
Frequency of Treatment:	7 h/day ; 5 d/week
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	Post-exposure period: none Control group: yes, concurrent no treatment Method: other: not specified

	Groups of male Sprague-Dawley rats (3l/group) were exposed whole-body to concentrations of 0, 2, 17 or 57 ppm methyl mercaptan for 7 hrs/day, 5 days/week for an overall period of 3 months. All animals were kept in closed colony cages (6 per cage) under controlled conditions of temperature and illumination for 1 wk before being committed to an experiment in order to screen for sick, suspicious, or overly aggressive types. A subset of 10 animals from each group was designated for special metabolic performance studies by an independent true random process. To minimize possible differences in feeding behavior during exposure periods, the sham and experimental groups were deprived of food during the exposure periods. Tap water was provided ad libitum. At the end of the exposure day the metabolic subsets were placed overnight in metabolism cages and the appropriate measurements were made. Metabolic performance measurements were made for 17 h periods on 5 consecutive days. At the end of the 3-mo experimental period the metabolic subsets served as the subjects for the following tests: intestinal transit time, systolic blood pressure effects, and histological examination of selected organs (heart, lungs, small intestine, liver, and kidneys). The observations were made at least 24 h later than the end of the last exposure day. Other biological data, obtained from the balance of the animals, included terminal body weight, 02 consumption, SMA 12160 blood analyses, and organ weights (brain, lung, liver, spleen, heart, kidneys, and adrenals). Complete histopathologies of livers of the 84 remaining sham and exposed rats were performed.

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL		=	17		ppm
LOAEL		=	57		ppm

Results Remarks:	No mortality response was observed in any sham or exposed population of rats during the 3-mo period. However, during actual exposures the rats tended to huddle in groups of 5 or 6 toward the periphery of the chamber with noses pointed outward from the chamber's vertical axis. This behavior was not observed in the sham group but was

	markedly obvious at 57 ppm. Average terminal body weights were lower than those of sham controls for all rats in the exposed groups. This difference was statistically significant in the 57 ppm group and showed a statistically significant dose-related trend. The same was true when average rates of body weight increases were determined by regression analyses for the metabolic subsets.. Although some average organ weights were significantly different from corresponding sham values, there was no obvious dose-related trend such as was apparent with whole-body weights, these significant differences could be due to chance alone. Average rates of change of food intake and wet and dry fecal weights were not significantly different from those of the sham controls. Fecal pellet production rate increases (data not shown) were significantly lower for the 2 and 17 ppm subsets and nonsignificantly greater for the 57 ppm subset. Rates of water intake increase were less for all exposed subsets, although this was not significant for the 57 ppm subset. Rates of water output increase were slightly higher for all exposed subsets, although the rate of increase for the 57 ppm subset was not significant. Statistically significant changes were observed in serum components of terminal blood samples from animals of all exposed groups subjected to SMA 12/60 analysis. Average total serum proteins were significantly higher for all exposed groups. Average albumin concentrations were significantly lower for all exposed groups. Significant reductions in inorganic phosphate occurred in the 2 and 17 ppm groups. Cholesterol was significantly elevated in the 2 ppm group and total bilirubin was significantly higher in the 2 and 17 ppm groups. Blood urea nitrogen was significantly lower in the 57 ppm group and lactate dehydrogenase was significantly lower in all three exposed groups. None of these trends were dose-related at the 95% confidence level. No significant differences in intestinal transit performance parameters were observed in any of the metabolic performance subsets. No consistent patterns were observed in average values of systolic blood pressure in the metabolic subset rats. After week 1, average values of oxygen consumption measured in special subsets tended to be lower for exposed than for sham rats, but the differences were not consistently significant. All of these average values tended to decrease with time during the limited course of the observations. However, for both the sham and exposed roups the mean oxygen consumption rates are higher than those expected for normal rats. These studies could not be conducted beyond 3 week because the rats grew too large to fit into the apparatus used for measuring oxygen consumption. Routine histopathological examination was conducted for five rats per dose group and was negative for the heart, small bowel, and kidneys. Lungs exhibited the pneumonia, emphysematic changes, and occasional fibrosis that are characteristic of rat colonies. These pictures did not appear to be different in samples from exposed rats. Some evidence of pathological changes was noted in liver sections from 31 rats each in the 2, 17, and 57 ppm groups. In all cases there was evidence of inflammatory cells and possibly enlarged bile ductules. Hyperplastic nodules were observed in one liver section from the 2 ppm and in thre e liver sections from the 57 ppm group. One hepatic

	carcinoma was visually observed (and sampled for histopathological examination) on the ventral surface of the liver of a rat in the 17 ppm group. In the case of the sham control group, 31 livers were hand-sectioned (2-3 urn) and examined under a dissecting microscope. Two small nodular lesions were detected in two livers; under light microscopy they were observed to be a hyperplastic region similar to that observed in the 2 and 57 ppm groups. Therefore, the treatment relationship of the hyperplastic nodules observed in the treated animals can be ruled out.
Conclusion:	LOAEL = 57 ppm NOAEL = 17 ppm
RELIABILITY/DATA QUALITY	
Reliability:	Valid With Restrictions; KS=2
Reliability Remarks:	Nonguideline study; adequate level of detail
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Tansy MF, Kendall FM, Fantasia J, Landin WE and Oberly R.1981. Acute and subchronic toxicity studies of rats exposed to vapors of methyl mercaptan and other reduced-sulfur compounds. J Toxicol Environ Health, 8, 71-88.

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]

High Production Volume Information System (HPVIS)

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	630-08-0
Test Substance:	Carbon Monoxide
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:	Repeated dose study in monkeys to determine cardiovascular effects of carbon monoxide exposure
Species:	Monkey
Other Species:	Not applicable
Mammalian Strain:	Cynomolgus monkeys *(Macaca irus irus)*
Other Strain:	Not applicable
Gender:	No data
Number of Animals per Dose:	6 -7
Concentration:	
Dose:	100 ppm
Year Study Performed:	1973
Method/Guideline Followed:	Other
GLP:	No data

Exposure Period:	3 months or 6 months
Frequency of Treatment:	23 hrs/day, continuously for 3 months or 6 months.
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	Control group: Yes, normal air. Method: To determine the cardiovascular effects of continuous exposure to carbon monoxide in monkeys. Normal monkeys and monkeys with induced myocardial infarction were continuously exposed to 100 ppm carbon monoxide for 24 weeks (23 hr/day) and the physiologic effects on the cardiovascular system were evaluated. Fifty·two animals (no gender information given) were randomly selected assigned to an air group or carbon monoxide group. Myocardial infarction was induced in 26 of these animals. Air·breathing and carbon monoxide breathing animals were housed in their respective chambers for one week prior to initiation of the carbon monoxide exposure procedures so they could acclimatize to their surroundings. Two exposure periods were used. In the first, the animals were continuously exposed to 100 ppm carbon monoxide (23 hr/day) for three months; in the other, the exposure period was extended to six months. The animals were divided into four groups: group 1 consisted of normal, air breathing animals; group 2 consisted of infarcted, air breathing animals; group 3 consisted of normal animals exposed to carbon monoxide; and, group 4 consisted of infarcted animals exposed to carbon monoxide. In the three·month exposure groups there were six animals in each group, and in the six·month exposure groups there were seven animals per group. During the exposure period blood was drawn every three weeks from each animal for chemical analyses and hematology; ECGs and body temperature were also recorded. The general well-being of the animals was assessed daily (alertness, food and water intake, stools, respiration, body weight). At the conclusion of the exposure period the animals were killed; gross observations were made on the heart and other tissues (brain, liver, spleen, kidney, lung, muscle, adrenals, and specimens were obtained for microscopic study.

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value	Value or Lower	Upper	Units:

		Description:	Concentration:	Concentration:	
LOAEL		=	100		ppm

Results Remarks:	When the animals were killed, histopathological changes consistent with those seen in human pathology following myocardial infarction were observed in the monkeys in which infarction was induced. Recognizable scarring and perivascular changes associated with the infarction occurred and significant and characteristic increases in the hematocrit, hemoglobin, and RBC levels were observed after three weeks exposure to 100 ppm carbon monoxide in both infarcted and noninfarcted animals. The changes persisted until the animals were killed three or six months later. The values of mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume (, platelets, blood cell count, fibrin split products, and serum enzymes (CPK, GOT, and LDH) did not change significantly during carbon monoxide inhalation. The ECGs of infarcted and noninfarcted animals breathing carbon monoxide displayed elevated P wave amplitudes. An analysis of variance of the P-wave amplitude changes (in millimeters) indicates that infarction was the source of significant variation during the early phase of the exposure period (ie, up to six weeks,$P < .025$ [F test]. By the 21st week of exposure the effect of infarction was minimal, presumably because healing was well advanced. Conversely, elevation of the P wave amplitude attributable to carbon monoxide inhalation was highly significant during the latter part of the exposure period (week 21, $P < 0.001$ [F test]). Of those animals which exhibited elevated P waves following exposure to carbon monoxide, four were randomly selected for histologic examination of the atria. Marked nuclear hyperplasia was observed in three, and minimal hyperplasia was seen in one, suggesting atrial hypertrophy. There was no pathological evidence of carbon monoxide-related changes in other tissues examined (i.e., brain, spleen, muscle, lung, kidney, adrenal).
Conclusion:	LOAEL = 100 ppm
RELIABILITY/DATA QUALITY	
Reliability:	Valid with Restrictions; KS=2
Reliability Remarks:	Non-guideline study; non standard species. Acceptable level of detail.
Key Study Sponsor Indicator:	Key
REFERENCE	

Reference:	DeBias DA, CM Banerjee, NC Birkhead, WV Harrer, LA Kazal.1973. Effects of carbon monoxide inhalation on ventricular fibrillation.Arch Environ Health 27: 161-167

High Production Volume Information System (HPVIS)

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	7664-41-7
Test Substance:	Ammonia
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:	Repeat dose
Species:	Rat

Other Species:	Not applicable
Mammalian Strain:	Wistar
Other Strain:	Not applicable
Gender:	Males 40-45 days old; 90-140 g
Number of Animals per Dose:	8-14 per group
Concentration:	
Dose:	0, 50 or 90 ppm (= 35 or 63 mg/m^3)
Year Study Performed:	1981
Method/Guideline Followed:	Other
GLP:	No data
Exposure Period:	50 days
Frequency of Treatment:	Continuous
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	Male Wistar rats were exposed to 0, 50 or 90 ppm ammonia. (control animals had no treatement) Clinical signs, body weight gain, food intake, haemoglobin, haematocrit, erythrocyte count, total and differential leucocyte count, total protein, lung- and liver weights were monitored. Statistical methods: Bartlett's test; Scheffe's test.
TEST RESULTS	

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL		=	90		ppm
NOAEL		=	50		ppm

Results Remarks:	LOAEL = 90 ppm NOAEL = 50 ppm Mortality: none Clinical signs: no treatment-related effects Body weight gain: 105 and 95% of the control for 50 and 90 ppm, respectively. Food intake: 94 and 108% of the control for 50 and 90 ppm, respectively. Haematology: increased haemoglobin, and haemotocrit comparable to control group at 90 ppm. Organ weights: no treatment-related effects.
Conclusion:	LOAEL = 90 ppm NOAEL = 50 ppm Based on body weight gain and food intake.
RELIABILITY/DATA QUALITY	
Reliability:	Unassignable; KS=4
Reliability Remarks:	Non standard test. Non standard test species
Key Study Sponsor Indicator:	Key
REFERENCE	

Reference:	Stolpe J, Sedlag R; 1976; Die Einzel- und Komplexwirkung von Ammoniak und Schwefelwasserstoff in der Luft auf kleine Versuchstiere (Ratten) bei unterschiedlichen Umweltbedingungen; Ach. Exper. Vet. Med. 30, 533-539.

High Production Volume Information System (HPVIS)

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	7783-06-4
Test Substance:	Hydrogen Sulfide
Test Substance Purity/Composition and Other Test Substance Comments:	
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 90 day study
Species:	Rat

Other Species:	Mouse
Mammalian Strain:	Sprague-Dawley and Fisher
Other Strain:	B6C3F1
Gender:	Both M/F
Number of Animals per Dose:	30 (15/sex/dose)
Concentration:	
Dose:	0, 10.1, 30.5, or 80.0 ppm
Year Study Performed:	1981
Method/Guideline Followed:	OECD 413
GLP:	Yes
Exposure Period:	90 Days
Frequency of Treatment:	6 hrs/day 5 days/wk for 90 days
Post-Exposure Period:	10 Days
Method/Guideline and Test Condition Remarks:	Post obs. period: 10 days following last exposure Control group: Yes, concurrent no treatment Method: A 90-day inhalation toxicity study using Fisher 344 rats, Sprague Dawley rats, and B6C3Fl mice (exposed simulateously in the same chamber) were conducted with H2S vapor. Three grps (15 male/15 female per grp) were designated as T-I, T-II, and T-III and exposed to atmospheres of 10.1, 30.5, and 80.0 ppm, respectively. In addition, control grps (15 male/15 female) were exposed to clean air only and

	were handled in a similar manner to that of the test animals. The duration of exposure was 6 hrs/day, 5days/wk, for at least 90 days.

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL		=	80 (Fisher rat; M-Sprague Dawley)		ppm
NOAEL		=	30 (F- Sprague Dawley; mouse)		
LOAEL		=	80 (F-Fisher rat; mouse)		ppm

Results Remarks:

NOAEL: 30 ppm (female SD rats; both male and female mice)
NOAEL: 80 ppm (male SD rats; both male and female Fisher rats)
LOAEL: 80 ppm (female SD rats; both male and female mice)

There was no mortality during the 90 day study. Clinical observations included crustiness associated with the animal's ear tag, crusty nose, eyes and muzzel, lacrimation, rales, yellow/brown stained fur and red stained fur. A significant decrease in body weight gains of all treatment groups of both sexes was noted after the first week of exposure. Body weights of the treated groups continued to lag behind the control group over the next 12 weeks. No significant changes were noted with respect to food consumption, opthalmology, nuerological function, clinical pathology, and organ weight data.

Gross and histopathologic studies did not reveal any lesions attributable to test article exposure. Special neuropathological studies preformed on teased fibers from muscular and sural branches of the tibial nerve, together with Epon embedded specimens from cervical and lumber spinal cord from control and high dose animals did not show neuropathologic changes.

Conclusion:	
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	CIIT. Toxigenics, Inc. 1983. 90-day vapor inhalation toxicity study of hydrogen sulfide in Fischer 344 rats. CIIT Docket No 22063. Chemical Industry Institute of Toxicology, ResearchTriangle Park, NC.

High Production Volume Information System (HPVIS)

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	No CAS number

<table>
<tr><td>Test Substance:</td><td>No CAS number</td></tr>
<tr><td>Test Substance Purity/Composition

and Other Test Substance Comments:</td><td>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
<table>
<tr><th>Component</th><th>Area %</th></tr>
<tr><td>Isobutane</td><td>2.70</td></tr>
<tr><td>n-butane</td><td>12.78</td></tr>
<tr><td>3-methyl-1-butene</td><td>0.41</td></tr>
<tr><td>Isopentane</td><td>36.50</td></tr>
<tr><td>n-pentane</td><td>9.36</td></tr>
<tr><td>Trans-2-pentene</td><td>3.60</td></tr>
<tr><td>2,3-dimethylbutane</td><td>1.75</td></tr>
<tr><td>2-methylpentane</td><td>7.25</td></tr>
<tr><td>3-methylpentane</td><td>4.27</td></tr>
<tr><td>n-hexane</td><td>3.62</td></tr>
<tr><td>Methylcyclopentane</td><td>1.87</td></tr>
<tr><td>2,4-dimethylpentane</td><td>1.36</td></tr>
<tr><td>Benzene</td><td>2.75</td></tr>
<tr><td>2-methylhexane</td><td>1.73</td></tr>
<tr><td>2,3-dimethylpentane</td><td>1.52</td></tr>
<tr><td>3-methylhexane</td><td>1.73</td></tr>
<tr><td>Isooctane</td><td>1.92</td></tr>
<tr><td>Toluene</td><td>3.91</td></tr>
</table></td></tr>
<tr><td>Category Chemical Result Type:</td><td>Measured</td></tr>
<tr><td>Unable to Measure or</td><td></td></tr>
</table>

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 24^0C 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.

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:	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:	5188-07-8
Test Substance:	Methanethiol, sodium salt
Test Substance Purity/Composition and Other Test Substance Comments:	31.4% sodium mercaptide solution in water (0.06% free NaOH).
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	

METHOD	
Type of Study:	Bacterial reverse mutation assay
Concentration:	

Concentrations:	312.5, 625, 1250, 2500 and 5000 ug/plate or 125, 250, 500, 1000 and 2000 ug/plate
Year Study Performed:	1992
Method/Guideline Followed:	OECD 471
GLP:	Yes
Positive, Negative and Solvent Control Substance(s):	Negative Control Substance Remarks: solvent control Positive contol: yes, but no available information.
Method/Guideline and Test Condition Remarks:	Type: Salmonella typhimurium reverse mutation assay Method: The in vitro potential mutagenic activity of sodium methyl mercaptide was investigated by the Ames test using 5 strains of bacteria Salmonella typhimurium: TA 1535, TA 1537, TA 102, TA 98 and TA 100. This test enables the detection of base-pair substitution and frameshift mutagens. After a preliminary assay to define the concentrations to be used for the mutagenicity study, 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, the S9 mix, prepared from a liver microsomal fraction S9 of rats treated with Aroclor 1254. The methods used were: - the direct plate incorporation method for the 2 assays without S9 mix and for the first assay with S9 mix, - the preincubation method (1 h, 37 deg C) for the second assay with S9 mix. The concentrations were: 312.5, 625, 1250, 2500 and 5000 ug/plate, except in the second test for the TA 98 and TA 102 strains without S9 mix: 1.25, 250, 500, 1000 and 2000 ug/plate, and for the TA 102 strain with S9 mix: 312.5, 625, 1250, 2500 and 4000 ug/plate. The negative and solvent control results were equivalent to those usually obtained in the Laboratory. The number of revertants induced by the positive controls was higher than the spontaneous one, which demonstrated the sensitivity of this test and the efficacy of the S9 mix throughout this study.

	Remark: The concentration above mentioned by the laboratory performing the study is related to the test article as such (sodium methyl mercaptan 31.4% w/w). However, the toxicity, variable in strains, make impossible to use higher concentrations. Vehicle used: Yes Vehicle Name: Unknown Metabolic Activation: With and Without Species or *in vitro* System: Salmonella typhimurium

TEST RESULTS

Details for Cytogenetic Assay (if applicable):	
Statistics:	

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 102	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 98	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 100	With and Without	Negative	Negative
Other Species:				

Other Strain:				

Results Remarks:	The test substance sodium methyl mercaptide did not induce any significant increase in the revertant number with or without S9 mix in any of the 5 strains. Cytotoxic Concentration: Without S9: >= 1000 ug/plate With S9: >= 2500 ug/plate
Conclusion Remarks:	Negative
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	ELF ATOCHEM. 1992. Sodium methyl mercaptide. Reverse mutation assay by the Ames test. CIT Report no. 9102 MO, 7 August 1992.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	5188-07-8

Test Substance:	Methanethiol, sodium salt
Test Substance Purity/Composition and Other Test Substance Comments:	21.21% sodium mercaptide solution in water (0.93% free NaOH)
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Type of Study:	*in vitro* mammalian chromosome aberration test
Concentration:	
Concentrations:	30, 60, 90, 120, 240, 480 ug/ml
Year Study Performed:	1983
Method/Guideline Followed:	OECD 473
GLP:	Yes
Positive, Negative and Solvent Control Substance(s):	Negative: yes Positive: yes
Method/Guideline and Test Condition Remarks:	Type: Chromosomal aberration test Method: Sodium methylmercaptide 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. For each culture, heparinised whole blood were added to culture medium containing a mitogen (phytohaemogglutinin) and incubated at 37°C.

	After 48 hours, the conditions of treatment were as follows, using 2 cultures/experimental point: -without S9 mix, the test or control substances remained in the culture medium either for 20 hours or for 44 hours, until harvest, i.e. approximately 1.5 times cell cycle or 24 hours after. -with S9 mix, the test or control substances remained in the culture medium for 3 hours. The cells were then rinsed and fresh culture medium was added. The cultures were then incubated either for 20 hours or for 44 hours, after the beginning of treatment until harvest, i.e. approximately 1.5 times cell cycle and 24 hours after. Each culture was then treated for 1.5 hours with a colcemid solution to block them at the metaphase-stage of mitosis and harvested. The chromosomal preparations were stained and screened microscopically for mitotic index and for aberrations: 200 well-spread metaphases per dose were read, whenever possible. After preliminary test, the cells were exposed to the following doses expressed as active material: 480, 240, 120, 90, 60 and 30 ug/ml. The top dose for scoring was selected according to the criteria specified in the international regulations. Since the test substance was toxic, the top dose was based on the level of toxicity: a toxic dose giving a reduction higher than 50% of mitotic index. Therefore, chromosome aberrations were scored on the slides corresponding to the following doses: without S9 mix: 30, 60, 120, 240 ug/ml, 1st harvest 30, 60, 90, 120 ug/ml, 2nd harvest. with S9 mix: 30, 60, 120, 240, 480 ug/ml, 1st harvest 30, 60, 120 ug/m l, 2nd harvest. Remark: The concentration above mentioned by the laboratory performing the study is related to the active material. Vehicle used: Yes Vehicle Name: No Data Metabolic Activation: With and Without
TEST RESULTS	
Details for Cytogenetic Assay (if applicable):	

Statistics:	

Species:	**Strain:**	**Metabolic Activation:**	**Genotoxic Effect:**	**Conclusion:**
Human lymphocytes		With and Without	Equivocal	Equivocal
Other Species:				
Other Strain:				

Results Remarks:	Sodium methylmercaptide did not induce structural chromosome aberrations both with and without S9 mix for both harvests. However, without S9 mix an increase in the number of polyploid cells was recorded at the 44-hour harvest at 90 and 120 ug/ml (4.0% and 14.5% respectively vs. 0%). Therefore, a complementary test without S9 mix at the 44-hour harvest was performed using the following doses: 50, 100 and 150 ug/ml. Since the mitotic index was reduced by more than 90% at 150 ug/ml, only slides from the 50 and 100 ug/ml treatment-level were scored. 3% polyploidy was noted at 100 ug/ml and 0% at 50 ug/ml. The frequencies of cells with structural chromosome aberrations of the vehicle and positive controls were as specified in acceptance criteria and within the range of the historical data for both tests and both harvest times. See attached robust summary document for a table of aberration frequencies. Genotoxic Effect: equivocal Cytotoxic Concentration: Without S9: >= 240 ug/ml With S9: >= 480 ugl/ml
Conclusion Remarks:	Sodium methylmercaptide did not induce structural chromosome aberrations but could induce numerical aberrations (polyploidy) in cultured human lymphocytes.

RELIABILITY/DATA QUALITY

Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study

Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	ELF Atochem. 1995. *In vitro* mammalian chromosome aberration test in cultured human lymphocytes with sodium methylmercaptide. CIT report no. 12086 MLH, 15 November 1995.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	75-08-1
Test Substance:	Ethanethiol (Ethyl mercaptan)
Test Substance Purity/Composition and Other Test Substance Comments:	"assumed 100%"
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Type of Study:	Bacterial reverse mutation assay
Concentration:	

Concentrations:	0, 123.5, 370.40, 1111.0, 3333.0, 10000 ug/plate
Year Study Performed:	1984
Method/Guideline Followed:	Other
GLP:	No data
Positive, Negative and Solvent Control Substance(s):	Solvent control: Dimethylsulfoxide (DMSO) Positive control: MNNG (without activation, TA 1535, TA 100) 9-Aminoacridine (without activation, TA 1537) 2-Nitrofluorene (without activation, TA TA 1538, TA 98) 2-Aminoanthracene (with metabolic activation)
Method/Guideline and Test Condition Remarks:	Type: Salmonella typhimurium reverse mutation assay Method: The in vitro potential mutagenic activity of ethyl mercaptan was investigated by the Ames test using 5 strains of bacteria Salmonella typhimurium: TA 1535, TA 1537, TA1538, TA 100, and TA 98. This test enables the detection of base-pair substitution and frameshift mutagens. Each assay was carried out both in the absence and in the presence of a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction of rats induced with Aroclor 1254. The samples were prepared in solutions for bioassay testing, with DMSO as the solvent. Samples were evaluated at five doses before mutagenicity testing with and without addition S9. Triplicate plates were evaluated at five doses as well as the solvent and positive controls. Criteria for a positive mutagenic response were defined as a mutagenic response at least twice the solvent control means. Solvent control: Dimethylsulfoxide (DMSO) Positive control: MNNG (without activation, TA 1535, TA 100) 9-Aminoacridine (without activation, TA 1537)

	2-Nitrofluorene (without activation, TA TA 1538, TA 98) 2-Aminoanthracene (with metabolic activation) Metabolic Activation: With and Without

TEST RESULTS

Details for Cytogenetic Assay (if applicable):	
Statistics:	2 times solvent control background

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	Slight increase	Negative (not dose-related)
Bacteria	S. typhimurium TA 1538	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 98	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 100	With and Without	Slight increase	Negative (not dose- related)
Other Strain:				
OtherSpecies:				

Results Remarks:	Exposure to five graded doses of ethyl mercaptan in the presence and in the absence of metabolic activation did not increase the reversion to histidine prototrophy of s. typhimurium strains 1535, 1538, or TA100. While slight increases were observed for strains 1537 and TA98,this response was not dose related. Cytotoxic concentration: 10,000 ug/plate

Conclusion Remarks:	Negative
RELIABILITY/DATA QUALITY	
Reliability:	Reliable Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline study in protocol and detail
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Hazleton Laboratories, Inc. 1984 (for Phillips Petroleum Co.). Salmonella typhimurium mammalian microsome plate incorporation assay with ethyl mercaptan, May 5, 1983. Project No. 652-145.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	75-08-1
Test Substance:	Ethanethiol (Ethyl mercaptan)
Test Substance Purity/Composition and Other Test Substance Comments:	97.5 % (adjusted to 100 %)

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:	25, 84, 250, 840, and 2500 ug/ml
Year Study Performed:	1984
Method/Guideline Followed:	Other
GLP:	No data
Positive, Negative and Solvent Control Substance(s):	Solvent control: dimethylsulfoxide (DMSO) Positive control: ethylmethanesulfonate (EMS) – without activation Positive control: cyclophosphamide – with activation
Method/Guideline and Test Condition Remarks:	Type: Sister chromatid exchange assay – CHO cells. Ethyl mercaptan 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. The maximum dose selected for the mutagenicity test was 2500 ug/ml because it exhibited growth inhibition. In the first test, all cells recovered at 2500 ug/l were first division metaphases which could not be analyzed for SCE's. A repeat test was peformed with 2500 ug/ml in which the chromosomes were recovered 43 hrs after exposure (instead of 24 hrs) in order to allow for two cell divisions. Solvent control: dimethylsulfoxide (DMSO) Positive control: ethylmethanesulfonate (EMS) – without activation Positive control: cyclophosphamide – with activation 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	Positive	Positive
CHO		Without	Positive	Positive
Other Species:				
Other Strain:				

Results Remarks:	In the absence of S9 mix, an increase of SCEs was observed at 840 ug/ml, the second highest concentration of ethyl mercaptan. Since the SCE's could not be analyzed at 2500 ug/ml (see Methods), another test was conducted at the 2500 ug/ml level. A statistically significant increase in SCE's was seen at 2500 ug/ml both with and without metabolic activation, and a greater than two-fold increase in SCE's was seen both with and without activation.
Conclusion Remarks:	Ethyl mercaptan is positive for inducing SCEs in cultured CHO cells 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:	Hazleton Laboratories, Inc. 1984 (for Phillips Petroleum Co.). *In vitro* sister chromatid exchange in Chinese hamster ovary cells. ethyl mercaptan. Final Report. December 11, 1984. OTS0571888.

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 1535	Rat/With - uninduced	Positive	Barely 2 fold above background at 30 % 1,3-butadiene in air.
Bacteria	S. typhimurium TA 1535	Mouse/ With -	Positive	Barely 2 fold above background at 30 % 1,3-

		uninduced		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.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	7664-41-7
Test Substance:	Ammonia
Test Substance Purity/Composition and Other Test Substance Comments:	Ammonia, purity: 99.9% (Takachiho Chemical Co. Tokyo, Japan.)
Category Chemical Result Type:	Measured

Unable to Measure or Estimate Justification:	
METHOD	
Type of Study:	Bacterial reverse mutation assay
Concentration:	
Concentrations:	0, 500, 1000, 2500, 5000, 10000 and 25000 ppm
Year Study Performed:	1985
Method/Guideline Followed:	Other
GLP:	No data
Positive, Negative and Solvent Control Substance(s):	Negative Control Substance Remarks: Air Positive control substances: 2- (2- Furyl) -3- (5-nitro-2-furyl) acrylamide (TA 100, TA 98 and *WP2uvrA* without S9) N-ethyl -N'-nitro -N – nitrosoguanidine *(TA1535 without S9)* 9-aminoacridine *(TA1537 without S9)* 4-nitroquinoline-l-oxide (TA 1538 without S9) benzo(a)pyrcnc (*TA100, TA98, TA1537 and TA1538 with S9)* 2-aminoanthracene *(TA1535 and WP2uvrA with S9)*
Method/Guideline and Test Condition Remarks:	Type: Salmonella typhimurium and *Escherichia coli* WP_2uvrA reverse mutation assay Method: The in vitro potential mutagenic activity of ammonia was investigated by the Ames test using 5 strains of bacteria Salmonella typhimurium: TA 1535, TA 1537, TA 1538, TA 98 and TA 100. This test enables the detection of base-pair substitution and frameshift mutagens. [Note that a total of 43 compounds were tested in this study] TEST SYSTEM - Species/cell type: *Salmonella typhimurium* and *Escherichia coli* - Deficiency/Proficiency: histidine and tryptophan - Metabolic activation system: Rat liver S9 mix (induced with polychlorinated biphenyl, KC500). ADMINISTRATION

	- Number of replicates: tests were performed in duplicate - In the test of such gaseous or high.ly volatile compounds as ammonia a minimal glucose agar plate covered with 0.1 ml of one of the strains and 0.5 ml of 89 mix or buffer was placed upside down withollt a lid inside a glass chamber. - Negative control group: air for ammonia - Positive control groups: see above - Exposure time: 48 hr DESCRIPTION OF FOLLOW UP REPEAT STUDY: Not described. CRITERIA FOR EVALUATING RESULTS - Statistical method: not indicated - method of calculation: not indicated

TEST RESULTS

Details for Cytogenetic Assay (if applicable):	
Statistics:	

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 1538	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 98	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 100	With and Without	Negative	Negative
Bacteria	Escherichia coli WP_2uvrA	With and Without	Negative	Negative

Other Species:				
Other Strain:				

Results Remarks:	The test substance ammonia did not induce any significant increase in the revertant number with or without S9 mix in any of the 5 strains of S. typhimurium or in Escherichia coli. Two chemicals tested (excluding the positive control, hexamethylenetetramine and 4,4'-methylenediphenyldiisocyanate, showed mutagenic activity in S. typhimurium TA98 and TA100 by metabolic activation. Hexamethylenetetramine also showed mutagenic activity in TA98 without microsomal activation. No mutagenic activity was observed in the ammonia group (or other 43 chemicals, 4 being gases). Cytotoxic Concentration: Without S9: Not indicated With S9: Not indicated
Conclusion Remarks:	Negative

RELIABILITY/DATA QUALITY

Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline study with sufficient level of experimental detail.
Key Study Sponsor Indicator:	Key

REFERENCE

Reference:	Shimizu H, Y Suzuki, N Takemura, S Goto and H Matsushita. 1985. The results of microbial mutation test for forty-three industrial chemicals, Jap J Ind Health 27, 400-419.

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	7783-06-4
Test Substance:	Hydrogen Sulfide
Test Substance Purity/Composition and Other Test Substance Comments:	"Highest quality available"
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Type of Study:	Bacterial reverse mutation assay
Concentration:	
Concentrations:	0, 17, 57, 175, 582, 1750 ug/plate
Year Study Performed:	1984
Method/Guideline Followed:	Other

GLP:	No data
Positive, Negative and Solvent Control Substance(s):	Solvent control: ethanol Positive control: Sodium azide (TA 100, 9-Aminoacridine (TA 97) 2-Nitrofluorene (TA 98 2-Aminoanthracene (TA 97, TA 98, TA100)
Method/Guideline and Test Condition Remarks:	Type: Salmonella typhimurium reverse mutation assay Method: The in vitro potential mutagenic activity of hydrogen sulfide was investigated by the Ames test using 3 strains of bacteria Salmonella typhimurium: TA 100, TA 98 and TA 97. This test enables the detection of base-pair substitution and frameshift mutagens. Each assay was carried out both in the absence and in the presence of a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction S9 of male Sprague Dawley rats treated with Aroclor 1254. Compounds were also tested with 5% male Syrian Golden hamster liver S9 . The protocol conditions were preincubation for 10 minutes at 37°C in full vials with no shaking during preincubation. The gaseous samples (four other gases tested in addition to hydrogen sulfide) were prepared in solutions for bioassay testing, with ethanol as the solvent. The gas concentration in each sample was determined by gas chromatography. Vapor-phase compounds were then tested in the Ames assay using a custom system developed and validated by the laboratory. Samples were first evaluated at five doses before mutagenicity testing with and without addition S9. Triplicate plates were evaluated at five doses as well as the solvent control. Criteria for a positive mutagenic response on strains TA97, TA98 and TA l00 were defined as a mutagenic response at two increasing dose levels that was at least twice the spontaneous background values. Solvent control: ethanol Positive control: Sodium azide (TA 100, 9-Aminoacridine (TA 97) 2-Nitrofluorene (TA 98 2-Aminoanthracene (TA 97, TA 98, TA100) Metabolic Activation: With and Without

TEST RESULTS	
Details for Cytogenetic Assay (if applicable):	
Statistics:	2 times background

Species:	Strain:	Metabolic Activation:	Genotoxic Effect:	Conclusion:
Bacteria	S. typhimurium TA 97	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 98	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 100	With and Without	Negative	Negative
Other Strain:				
OtherSpecies:				

Results Remarks:	
Conclusion Remarks:	The test substance hydrogen sulfide did not induce any significant increase in the revertant number with or without S9 mix in any of the 3 strains (either rat or hamster activation system) Cytotoxic Concentrations: not identified. High test concentrations of H2S were difficult to obtain in liquid suspension, and were limited by solubility of the test gas in the test solvent. H2S was soluble in ethanol at a high dose of 1, 750 ug/plate. Consequently, the authors recommend that additional experiments should be conducted with these vapors in the gaseous state, where much higher dose levels can be attained.
RELIABILITY/DATA QUALITY	
Reliability:	Reliable With restrictions (KS=2)

Reliability Remarks:	Non guideline study; only three strains of bacteria used; limited solubility of H2S
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Hughes, T., C. Sparacino, and S. Frazier. 1984. Validation of chemical and biological techniques for evaluation of vapors in ambient air/mutagenicity testing of twelve (12) vapor-phase compounds. Research Triangle Park, NC: U.S. Environmental Protection Agency, Health Effects Research Laboratory. EPA/600/1-84/005. (NTIS PB84164219).

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:	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	The test material was dissolved in acetone for this assay. The positive control substances were Ethyl methane sulphonate (EMS) and Dimethylnitrosamine (DMN).

and Test Condition Remarks:	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	L5178Y TK+/-	With	Negative	Negative

lymphoma				
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 (µl/ml)	**Rel. susp. growth**	**Mutant clones**	**Viable clones**	**% Rel. growth**	**Mutant 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
	<u>Activation</u>					
	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.

Genetic Toxicity *in vitro*

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:	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 (if applicable):	**Details for Cytogenetic Assay (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

Dose level	Salmonella strains TA100	TA1535		TA1537		Yeast 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.

Genetic Toxicity *In Vivo*

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	No data

Comments:	
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.

Genetic Toxicity *in vivo*

TEST SUBSTANCE	
Category Chemical:	74-93-1
Test Substance:	Methanethiol
Test Substance Purity/Composition and Other Test Substance Comments:	No data
Category Chemical Result Type:	Measured
METHOD	
Type of Study:	In vivo mouse micronucleus assay
Type of Test:	
Route of Administration:	Inhalation
Species:	Mouse
Strain:	Swiss Webster
Gender:	Both M/F

Dose:	0, 114, 258, or 512 ppm
Year Study Performed:	1983
Method/Guideline Followed:	OECD 474
GLP:	Yes
Duration of Treatment/Expososure Period and Units:	6 Hours
Frequency of Treatment:	Single dose
Positive, Negative and Solvent Control Substance(s):	Positive Control Substance Remarks : urethane Negative Control Substance Remarks: air
Post-Exposure Period:	
Number of Animals per Sex per Dose:	15
Method/Guideline and Test Condition Remarks:	Type: Micronucleus assay The genotoxic potential of nose-only inhalation exposure of methyl mercaptan to induce micronucleus formation in bone marrow erythrocytes was determined in Swiss-Webster mice. In the dose-range finding study, three mice per sex per treatment group received a single 6-hour nose-only inhalation exposure to methyl mercaptan at 112, 374, and 570 ppm. A control group, consisting of three male and three female mice, received air only. Mice were observed daily from the start of treatment until death or sacrifice. The concentration ranges for the low- and mid-concentrations exceeded the protocol criterion of 10%. These deviations are judged not to have had a significant adverse effect on the study. In the definitive experiment, 15 mice per sex per treatment group were exposed to methyl mercaptan by nose-only inhalation at 114, 258, or 512 ppm. Five mice per sex per group were sacrificed 24,48, and 72 hours cytotoxicity and micronucleus formation. An air-exposed control group of male and female mice and a urethane positive control group of male mice were treated similarly and evaluated concurrently with the methyl mercaptan-treated groups. Vehicle used: No Data

	Mammalian Strain: Swiss Webster
TEST RESULTS	
Systemic Toxicity:	
Genotoxic Effect:	Negative
Results Remarks:	DOSE RANGE FINDING EXPERIMENT No significant differences as observed between terminal and pre-exposure body weights of each of the treatment groups at each of the sacrifice times. Clinical signs observed included shallow breathing at the fourth hour of exposure at 112 ppm, shallow breathing at the third hour of exposure at 374 and 570 ppm with hypoactivity at the mid and high dose levels in all mice when observed after completion of exposure. Two male mice were found dead near the end of the second hour and during the sixth hour of exposure at 570 ppm. Any mouse showing clinical signs appeared normal on Day 2. Surviving mice were sacrificed approximately 72 hours after the inhalation exposure, and cytotoxicity was determined based on the ratio of RNA-positive erythrocytes (PCEs) to total red blood cells (RBCs) in both peripheral blood and bone marrow smears. No significant PCE suppression was observed in any of the methyl mercaptan treatment groups when compared to the air control group in either peripheral blood or bone marrow. DEFINITIVE EXPERIMENT Clinical observations in this experiment included shallow breathing and hypoactivity at the fourth and fifth hours, respectively, of exposure at 258 ppm in all mice. All mice at 258 ppm appeared normal on Day 2 and on all subsequent experiment days. Shallow breathing at the third and fourth hours of exposure, and hypoactivity at the fifth hour of exposure were observed at 512 ppm in all mice. One female mouse was found dead after 2 hours of exposure at 512 ppm, and two female and two male mice were found dead at 512 ppm on Day 2. All surviving mice at 512 ppm appeared

normal on Day 2 and on all subsequent experiment days. Mice treated with 114 ppm methyl mercaptan, air control, or urethane appeared normal throughout the experiment. The percentages of PCEs among RBCs in groups treated with methyl mercaptan did not differ significantly fro m those of the air control groups in any of the dose groups for either sex.
In male mice, none of the individual dose groups had a statistically significant increase in MN frequency. Using the Cochran-Armitage test for a trend in binomial proportions, a statistically significant upward trend in micronucleus (MN) frequency was observed in female mice sacrificed at 24 hr after exposure to the methyl mercaptan.
However, the MN frequency in the control group was lower than the laboratory historical value (0.21%) for females of this strain of mice, and none of the individual dose groups had a statistically significant increase in MN frequency.

DEFINITIVE EXPERIMENT IN MALE SWISS-WEBSTER MICE TREATED WITH A SINGLE EXPOSURE OF METHYL MERCAPTAN: MICRONUCLEUS FREQUENCY

Dose (ppm)	Time (hrs)	nb	PCE/RBC(%) Mean±S.E.	PCE with MN Mean±S.E.
0.0	24	5	57.10±4.18	0.13±0.02
114.0	24	5	57.61±5.09	0.15±0.03
258.0	24	5	52.27±7.70	0.16±0.02
512.0	24	5	45.46±5.45	0.18±0.02
Urethane	24	5	52.84±5.69 0	0.65±0.10
0.0	48	5	51.31±4.83	0.18±0.05
114.0	48	5	52.93±6.80	0.13±0.03
258.0	48	5	47.05±3.88	0.17±0.02
512.0	48	5	49.25±2.22	0.17±0.03
Urethane	48	5	40.09±3.66	0.45±0.11*
0.0	72	5	52.76±4.12	0.10±0.03

114.0	72	5	44.16±5.90	0.15±0.04
258.0	72	5	42.56±6.87	0.09±0.01
512.0	72	3	52.35±2.47	0.18±0.03
Urethane	72	5	42.54±3.08	0.22±0.02*

* Statistically different from control ($p < 0.01$) by test for binomial proportions.

DEFINITIVE EXPERIMENT IN MALE SWISS-WEBSTER MICE
TREATED WITH A SINGLE EXPOSURE OF METHYL MERCAPTAN:
MICRONUCLEUS FREQUENCY

Dose (ppm)	Time (hrs)	nb	PCE/RBC(%) Mean±S.E.	PCE with MN Mean±S.E.
0.0	24	5	47.39±4.67	0.09±0.02
114.0	24	5	52.83±4.60	0.10±0.03
258.0	24	5	53.68±3.04	0.12±0.03
512.0	24	5	49.98±2.07	0.17±0.04
0.0	48	5	49.06±2.24	0.12±0.05
114.0	48	5	56.58±2.60	0.13±0.04
258.0	48	5	58.73±3.76	0.13±0.02
512.0	48	5	50.07±3.96	0.17±0.05
0.0	72	5	59.41±7.54	0.10±0.04
114.0	72	5	55.66±4.10	0.13±0.04
258.0	72	5	52.46±3.85	0.12±0.03
512.0	72	3	51.96±4.98	0.16±0.04

Conclusion:	negative
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study

Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Elf Atochem North America. 1997. Bone marrow micronucleus assay in male and female Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan. SRI International report no. M020-95, 8 January 1997.

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 and Other Test Substance Comments:	No data
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.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vivo*

TEST SUBSTANCE	
Category Chemical:	7664-41-7
Test Substance:	Ammonium chloride

Test Substance Purity/Composition and Other Test Substance Comments:	Purity 99.7 Ammonium chloride
Category Chemical Result Type:	Measured
METHOD	
Type of Study:	In vivo mouse micronucleus assay
Type of Test:	
Route of Administration:	i.p. injection
Species:	Mouse
Strain:	ddY
Gender:	Male
Dose:	0, 62.5, 125, 250, 500 mg/kg – single dose 0, 31.3, 62.5, 125, 250 mg/kg – 4 doses
Year Study Performed:	1988
Method/Guideline Followed:	Other
GLP:	No data
Duration of Treatment/Expososure Period and Units:	Single dose, i.p 4 doses at 24 –hr intervals
Frequency of Treatment:	Single dose; 4 doses

Positive, Negative and Solvent Control Substance(s):	Positive Control Substance Remarks: Mitomycin C Negative Control Substance Remarks: Saline
Post-Exposure Period:	
Number of Animals per Sex per Dose:	6 per dose level
Method/Guideline and Test Condition Remarks:	Type: Micronucleus assay The genotoxic potential of i.p. exposure to ammonium chloride to induce micronucleus formation in bone marrow erythrocytes was determined in Swiss-Webster mice. Thirty eight other materials were evaluated at the same time, however only the ammonium chloride results are reported here. The maximum dose of ammonium chloride was determined by pilot experiments using the multisampling at multi-dose levels method. Doses up to MTD (maximum tolerance dose) were administered. In one experiment, mice were killed 24 hr after an administration. Femoral marrow cells were flushed out with fetal bovine serum and fixed with methanol and stained with Giemsa. One thousand polychromatic erythrocytes per mouse were scored using a light microscope and the number of micronucleated erythrocytes was recorded. In a second experiment, multiple injections were given, with 24 hr intervals between injections, and killed 24 hr after the last injection. Cells were prepared the same as in the first experiment. A two stage statistical procedure was used. In the first step, the frequency of the micronucleated erythrocytes was compared with the binomial distribution specified by historical control data from the laboratory. In the second step, the dose-response relationship was tested by the Cochran-Armitage trend test. A positive result was recorded only when one or more treatment group(s) showed a statistically significant difference ($P<0.01$) from the spontaneous level of micronucleated erythrocytes and the trend test indicated a positive dose-response. . Positive Control Substance Remarks: Mitomycin C Negative Control Substance Remarks: Saline
TEST RESULTS	
Systemic Toxicity:	
Genotoxic Effect:	Negative
Results Remarks:	DEFINITIVE EXPERIMENT No mortality occurred following ammonium chloride administration. No clinical observations were evaluated in this study.

The percentages of PCEs among RBCs in groups treated with ammonium chloride did not differ significantly from those of the saline control groups in any of the dose groups.

MICRONUCLEUS FREQUENCY IN MALE MICE TREATED WITH A SINGLE EXPOSURE OF AMMONIUM CHLORIDE

Dose (mg/kg)	Time (hrs)	nb	PCE/RBC(%) Mean±S.E.	PCE with MN Mean±S.E.
0.0	24	6	56.8±4.7	0.18±0.18
62.5	24	6	60.9±4.2	0.12±0.12
125	24	6	61.7±3.8	0.15±0.14
250	24	6	64.3±2.5	0.13±0.05
500	24	6	56.9±6.1	0.12±0.08
Mytomycin C (2.0)	24	6	52.3±4.6	4.18±1.05*

* Statistically different from control ($p < 0.01$) by test for binomial proportions.

MICRONUCLEUS FREQUENCY IN MALE MICE TREATED WITH 4 DOSES OF AMMONIUM CHLORIDE AT 24-HR INTERVALS

Dose (mg/kg)	Time** (hrs)	nb	PCE/RBC(%) Mean±S.E.	PCE with MN Mean±S.E.
0.0	24	6	59.9±8.3	0.20±0.09
31.3	24	6	67.2±13.5	0.25±0.19
62.5	24	6	63.7±4.5	0.17±0.10
125	24	6	64.0±9.2	0.20±0.18
250	24	6	61.6±6.9	0.17±0.08
Mytomycin C (2.0)***	24	6	32.2±11.0	7.15±3.92*

* Statistically different from control ($p < 0.01$) by test for binomial

	proportions. ** Time after last injection *** Single dose administered
Conclusion:	negative
RELIABILITY/DATA QUALITY	
Reliability:	Valid With Restrictions (KS=2)
Reliability Remarks:	Nonguideline study; single sex studied
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Hayashi M, Kishi M, Sofuni T and M. Ishidate Jr. 1988. Micronucleus test in mice on 39 food additives and eight miscellaneous chemicals. Food. Chem.Toxicol. 26, 487-500.

High Production Volume Information System (HPVIS)

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 20^{th} 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.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vivo*

TEST SUBSTANCE	
Category Chemical:	No CAS number
Test Substance:	No CAS number
Test Substance	C5-C6

Purity/Composition and Other Test Substance Comments:

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 20^{th} 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.

Reproductive Toxicity

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:	75-28-5
Test Substance:	Isobutane
Test Substance Purity/Composition and Other Test Substance	99.0% (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.

Comments:	
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 (with added neurotoxicity evaluation)
Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	24 (12/sex/dose level)
Concentration:	
Dose:	0, 900, 3000 and 9000 ppm
Year Study Performed :	2004

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: concurrent air only Premating exposure period: Male-2 weeks Female- 2 weeks Method: The reproductive toxicity of isobutane was assessed in an OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. Neurotoxicity was also evaluated. Isobutane was administered as a gas to Sprague Dawley CD rats (12/sex/dose in main study group and 12 females/dose in the satellite group) at target concentrations of 900, 3000 and 9000 ppm (note: highest dose is 50% of the lower explosive limit) 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/dose group) were exposed once daily (6 hours/day), 7 days/week for 4 weeks (minimum of 28 days). Satellite female rats 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. Main study females were evaluated for subchronic effects and satellite females for reproductive effects only. The following parameters were evaluated in all animals: viability, clinical observations, body weights, feed consumption, functional observational battery (FOB), motor activity, clinical pathology (termination), organ weights, and macroscopic observations. Microscopic pathology was conducted in the main study control and high-exposure groups only).
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 (systemic)	=	9000		ppm
LOAEL	Reproductive	=	9000		ppm
NOAEL	Reproductive	=	3000		ppm
NOAEL	Developmental	=	9000		ppm

Results:

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.

No general systemic/neurotoxic effects were observed. A no-observed-adverse effect level (NOAEL) of 9000 ppm was determined for all general systemic/neurotoxic 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. The NOAEL for pup endpoints was 9000 ppm based on no effects in offspring survival, body weight and development up to post-natal day.

Results Remarks:	
Conclusion:	Parental Systemic NOAEL (Sprague Dawley rats) = 9000 ppm Reproductive LOAEL = 9000 ppm Reproductive NOAEL = 3000 ppm Developmental NOAEL (Sprague Dawley rats) = 9000 ppm
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, reproduction and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Insitute. Draft report 03-4244.

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:	630-08-0
Test Substance:	Carbon monoxide
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:	
Type of Exposure:	Fetal effects study
Species:	Rat
Other Species:	

Mammalian Strain:	Long-Evans
Other Strain:	
Gender:	Female
Number of Animals per Dose:	56 total pregnant females were assigned to four groups at 12-16 animals each – no further detail given.
Concentration:	
Dose:	0, 30 and 90 ppm
Year Study Performed :	1978
Method/Guideline Followed:	Other
GLP:	No data
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** Continuous
Frequency of Treatment:	7 days/week, gestation days 3 to 20
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	Duration of test: Females - gestation day 3-20 Control group: yes, concurrent room air treatment Method: To investigate the effects of continuous exposure to carbon monoxide on fetal growth and development. 56 (sperm positive) Long-Evans rats were divided into 4 experimental groups and placed in sealed exposure chambers. The four groups consisted of 12-16 animals each, inspired the following gas mixtures: 1) room air (controls); 2) air with 30 ppm carbon monoxide: 3) air with 90 ppm carbon monoxide and 4) 13% O2 in

	nitrogen. Animals were exposed day 3 of gestation until 2 days prior to term(day 20 of gestation). The following information was recorded: litter size, the number of fetuses alive, the number dead and the number visibly abnormal. The fetuses were weighed in rapid succession, decapitated and trunk blood was collected for carboxyhemoglobin determination. A maternal blood sample from the inferior vena cava was also collected and the PO2 , PCO2, pH ,the hemoglobin concentration, oxyhemoglobin saturation, and the carboxyhemoglobin was measured, as well as the hematocrit and counts of red blood cells and reticulocytes. The placenta and various fetal organs (brain, heart, lungs, liver, kidneys and *en bloc* the stomach-intestine-spleen-pancreas (SISP) were weighed. The fetal brains were quickly frozen on dry ice and stored for analysis of protein concentrations and measurements of the concentrations of 2 neurotransmitters-norepinephrine and serotonin. The data from each individual maternal rat and her fetuses were treated separately, using Student's t test to determine the level of significant difference between the control and the treated animals.
Pre-Mating Exposure / Males :	
Pre-Mating Exposure / Females:	None

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Maternal, systemic	=	30		ppm
LOAEL	Reproductive	=	30		ppm

Results:	There was a marked reduction in the number of successful pregnancies as determined by the number of sperm-positive animals that were with litter on day 20 of gestation. The percentages of successful pregnancies/group were: control, 100%; 30 ppm carbon monoxide, 69%; 90 ppm carbon monoxide, 38% and 13% O2, 50%. No visible resorption sites at the time of sacrifice of any of the sperm-positive, nonpregnant animals. Therefore, the authors

	believe that failure of blastocyst implantation accounted for the majority of the unsuccessful pregnancies. Neither of the carbon monoxide-exposed groups showed a hematologic response, a result suggesting that the concentrations used in this experiment remained below the threshold level needed to produce such effects. The 13% O2 group exhibited an increased hematocrit, erythrocyte count and hemoglobin concentration in response to the hypoxia. The 90 ppm group did, however, show a decreased venous PCO2 which accompanied a slight (but not statistically significant) alkalosis; these changes may have resulted from hyperventilation by these animals. Exposure to 30 and 90 ppm carbon monoxide resulted in maternal carboxyhemoglobin concentrations, of 4.8 ± 0.3% and 8.8 ± 0.7%, respectively. Both were statistically different than the control values of 0.6±0.1 %. Under steady state conditions, the carboxyhemoglobin concentration of fetal blood, is somewhat higher than the maternal levels (11.3 ± 0.7% for 30 ppm and 17.0 ± 1.0% for 90 ppm). The ratio of fetal to maternal carboxyhemoglobin reflects both the relative affinities of maternal and fetal blood for O2 and the relative affinities of each type of hemoglobin for carbon monoxide as compared with O2. The mean fetal/maternal carboxyhemoglobin ratio equaled 2.1. The average number of fetuses/litter for each group were: control, 7.6; 30 ppm carbon monoxide, 10.2; 90 ppm carbon monoxide, 8.8 and 13% O2 , 7.8. Comparison of the average litter size may be misleading in as much as the average number/litter for the control group was somewhat lower than the averages of 10 or more frequently reported for this strain of animals. No differences in the number of fetuses alive, recently dead or visibly abnormal in any of the treated animals were found. Only the 13% O2 group demonstrated a decrease in fetal body weight. This decrease may reflect the 26% reduction in food consumption found in this group. Neither carbon monoxide exposed group exhibited a significant decrease in food consumption or body weight. No significant differences in wet weights of individual fetal organs between the 30 ppm carbon monoxide group and control group were detected. However, in the 90 ppm carbon monoxide group, the brain wet weight was increased 14% while the lungs were decreased 24%. No significant differences appeared between any of the experimental groups and controls when results were expressed as brain to body weight ratio. The fetal lung and liver weights of the 13% O2 group were lower than the control values; however, statistically significant differences are lost for both organs if the values are expressed as organ to body weight ratios. Conversely, a significant difference appears if the placental weight of this group is expressed as placenta/body weight. In regard to fetal brain development, at 30 ppm carbon monoxide, no significant effect was seen in any of the parameters measured, i.e., total content of protein, DNA, or serotonin or norepinephrine as compared to the control. The 90 ppm carbon monoxide group showed a marked reduction in the concentrations of protein, DNA, and serotonin. Norepinephrine concentration difference between controls and the 90 ppm carbon monoxide group was not statistically significant. According to the authors, the dilution of the concentrations of the brain constituents for the 90 ppm carbon monoxide group appeared to result from an increased brain water content (edema) because the

	concentrations of all constituents were similar to control values when expressed in terms of content/brain. They also indicate that the lower dry weight percentage for the brains of this group also supports the presence of brain edema in the 90 ppm carbon monoxide fetuses. The pathophysiologic repercussions of the brain edema in the 90 ppm carbon monoxide fetuses were not determined by this experiment. However, the existence of edema in this organ at a time of rapid growth and maturation may be important.
Results Remarks:	
Conclusion:	NOAEL(Maternal) = 30 ppm LOAEL(reproductive) = 30 ppm; no NOAEL identified – based on decreased percent of pregnant females.
RELIABILITY/DATA QUALITY	
Reliability:	Valid With Restrictions (KS=2)
Reliability Remarks:	Non guideline study. Only two doses used; some detail lacking
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Garvey, DJ and LD Longo. 1978. Chronic low level maternal carbon monoxide exposure and fetal growth and development. BioI. Reprod. 19:8-14.

High Production Volume Information System (HPVIS)

Reproductive Toxicity

TEST SUBSTANCE	
Category Chemical:	7664-41-7

Test Substance:	Ammonia
Test Substance Purity/Composition and Other Test Substance Comments:	Ammonia (NH_3) obtained by flushing of manure pits weekly.
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	
Type of Exposure:	Inhalation of ammonia in air environment
Species:	Pig
Other Species:	
Mammalian Strain:	Yorkshire x Hampshire x Chester White gilts (a young sow)
Other Strain:	
Gender:	Females only
Number of Animals per Dose:	40
Concentration:	
Dose:	7 ppm (=4.9 mg/m^3) and 35 ppm (=24.3 mg/m^3) [both values were means]

Year Study Performed :	1993
Method/Guideline Followed:	Other
GLP:	No data
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** continuous
Frequency of Treatment:	6-weeks pre-mating; 10-weeks post-mating
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	Type: one generation Duration of test: 6 weeks prior to mating, 10 weeks post mating. Control group: none From 2-4.5 months of age, gilts were exposed naturally to Mycoplasma hyponeumoniae and Pasteurella multocida, which cause enzootic pneumonia and atrophic rhinitis, respectively. At 4.5 months of age, the gilts were moved to one of two rooms and exposed to either low (mean 7 ppm) or moderate (mean 35 ppm) aerial concentrations of ammonia. Each exposure group consisted of 40 individuals. In the room with low ammonia concentration, manure was flushed weekly to maintain a 0.3 m depth. In the room with moderate ammonia concentration, manure accumulated to 0.48 m depth. Moderate aerial ammonia concentration was obtained initially and maintained by adding anhydrous ammonia from a steel tank. Mean daily gain was determined by weighing the gilts biweekly. Half the gilts from each exposure concentration were sacrificed after 6 weeks. The remaining gilts were maintained in their respective environments, exposed daily to mature boars, bred at first estrus, and sacrificed at day 30 of gestation.
Pre-Mating Exposure / Males :	Not exposed
Pre-Mating Exposure / Females:	6 weeks
TEST RESULTS	
Concentration (LOAEL/LOAEC/NOAEL/NOAEC)	

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Parental (F0)	=	35		ppm
NOAEL	Offspring (F1)	=	35		ppm

Results:	At the end of two weeks, gilts in the moderate exposure group weighed less than those in the low exposure. After 2 weeks gilts acclimated and the mean daily gain was similar for the rest of the experiment. The gilts sacrificed at 6 weeks showed that the animals in the low exposure were heavier. At day 30 of gestation, number of live fetuses, fetal length, and fetus-to-corpus luteum ratio were all similar between the two groups. No statistically significant differences were noted in ovarian or uterine weights of pigs exposed to about No unexposed controls were included in the study. No statistically significant difference in fetal length was evident at 30 days of gestation in offspring of pig dams that were continuously exposed to about 7 or 35 ppm ammonia from 6 weeks before breeding until day 30 of gestation (Diekman et al. 1993).
Results Remarks:	
Conclusion:	NOAEL = 35 ppm
RELIABILITY/DATA QUALITY	
Reliability:	Not assignable (KS=4)
Reliability Remarks:	Non guideline; nonstandard species; lack of detail; no controls
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Diekman, MA., Scheidt, AB., Sutton, AL., Green, ML., Clapper, JA, Kelly, DT., and Van Alstine, WG. 1993.

	Growth and reproductive performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. Am J Vet Res 54(12):2128-2131.

Reproductive Toxicity

TEST SUBSTANCE	
Category Chemical:	7783-06-4
Test Substance:	Hydrogen sulfide
Test Substance Purity/Composition and Other Test Substance Comments:	Hydrogen sulfide supplied by Holox gases (Cary, NC)
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/dose level)
Concentration:	
Dose:	0, 10, 30, 80 ppm
Year Study Performed :	1995
Method/Guideline Followed:	OECD 421
GLP:	Yes
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** 6 hrs/day
Frequency of Treatment:	7 days/week
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, concurrent no treatment Method: This study investigated the effects of perinatal exposure by inhalation to hydrogen sulfide (H2S) on pregnancy outcomes, offspring prenatal and postnatal development, or offspring behavior. Virgin male and female Sprague-Dawley rats (12 rats/sex/concentration) were exposed (0, 10, 30, or 80 ppm H2S; 6h/day, 7 days/week) for 2 weeks prior to breeding. Exposures continued during a 2-week mating period (evidence of copulation = gestation day 0) and then from gestation day 0 through gestation day 19. Exposure of dams and their pups (eight rats/litter after culling) resumed between postnatal day 5 and 18. Adult male rats were exposed for 70 consecutive days. Offspring were evaluated using motor activity (postnatal day 13, 17, 21, and 60 +/- 2), passive avoidance (postnatal day 22 +/- 1 and 62 +/- 3), functional observation battery (postnatal day 60 +/- 2) acoustic startle response (postnatal day 21 and 62 +/- 3) and neuropathology (postnatal day 23 +/- 2 and 61 +/- 2).
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)	=	80		ppm
NOAEL	Offspring (F1)	=	80		ppm

Results:	There were no deaths and no adverse physical signs observed in F0 male or female rats during the study. A statistically significant decrease in feed consumption was observed in F0 male rats from the 80 ppm H2S exposure

	group during the first week of exposure. There were no statistically significant effects on the reproductive performance of the F0 rats as assessed by the number of females with live pups, litter size, average length of gestation, and the average number of implants per pregnant female. Exposure to H2S did not affect pup growth, development, or performance on any of the behavioral tests.
Results Remarks:	
Conclusion:	NOAEL = 80 ppm
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Dorman, DC, MF Struve, EA Gross and KA Brenneman. 2000. Fertility and developmental neurotoxicity effects of inhaled hydrogen sulfide in Sprague-Dawley rats. Neurotoxicology and Teratology. 22:71-84.

High Production Volume Information System (HPVIS)

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	Baseline Gasoline Vapor Condensate was administered via whole-body exposures to Sprague Dawley rats over 2

and Test Condition Remarks:	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. <u>Parturition and Lactation</u>: 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. <u>Pups</u> (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. <u>Pup data (F1 & F2 generations)</u>: 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:	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.

DEVELOPMENTAL TOXICITY/TERATOGENICITY

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)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	630-08-0
Test Substance:	Carbon monoxide
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:	
Type of Exposure:	Developmental toxicity study
Species:	Mouse
Other Species:	
Mammalian Strain:	CD-1
Other Strain:	
Gender:	Females
Number of Animals per Dose:	No data
Concentration:	
Dose:	0, 65, 125, 250, or 500 ppm

Year Study Performed :	1984
Method/Guideline Followed:	Other
GLP:	No data
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** continuous
Frequency of Treatment:	7 days/week, gestation days 7-18
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	Control group: yes, concurrent compressed air Method: This study investigated the effects of carbon monoxide exposure on pregnancy and fetal outcomes. Female albino CD-1 mice were bred overnight with males of the same strain, and the day a copulation plug was found was designated as gestation day 1. The pregnant animals were exposed continuously to 0, 65, 125, 250, or 500 ppm carbon monoxide in air in plexiglas environmental chambers from gestation day 7 to 18. The concentration of carbon monoxide was monitored at each chamber inlet by carbon monoxide detector. The animals were killed on gestation day 18, and their uterine horns were examined for gross malformations. One-third of the fetuses were examined for skeletal abnormalities. Litter means for fetal weight, number of live fetuses, and number of dead or resorbed fetuses were used to test for carbon monoxide effects on weight and fetal mortality. Analysis of variance and Student·Neuman-Keuls multiple range tests were used for the comparison between control and test groups for fetal weights and mean number of dead or resorbed fetuses per litter. Mean percent fetal mortality was obtained by calculating the mean of litter means. Results are based on data from 17 litters for each concentration of carbon monoxide. Further studies using the same experimental protocol, but at exposure levels of 0, 65, or 125 ppm carbon dioxide from day seven to 18 of pregnancy examined its effects on neonatal reflex development. (Singh, 1986) COHb was not not determined in either study.

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Female (Maternal)	=	500		ppm

NOAEL	Offspring (F1)- fetal weight	=	65		ppm
LOAEL	Offspring (F1) – fetal weight	=	125		ppm

Results Remarks:	No sign of maternal toxicity was observed under the conditions of exposure. However, effects were observed in the offspring. The mean percent fetal mortality per litter from mothers exposed to carbon monoxide at 0, 65, 125, 250, or 500 ppm was 4.52, 5.89, 12.50, 15.50, and 55.30, respectively. The mean number of dead or resorbed fetuses per litter in the high-dose group was significantly greater than the control value. Weights of fetuses from 125, 250, and 500 ppm carbon monoxide-exposed mothers were significantly decreased when compared to weights of controls. Fetal weight was not significantly influenced by 65 ppm carbon monoxide exposure, although the decreased value compared to controls was suggestive of an effect. A small number of skeletal anomalies (lack of ossofication) were observed in fetuses from all groups, however, these anomalies were not dose dependent. Data suggest that maternal carbon monoxide exposure to as low as 125 ppm can affect fetal growth and that higher levels impair viability. The fetus appears to be sensitive to chronic carbon monoxide exposure and this sensitivity is dose dependent. Note: see additional data from another study done in this laboratory (Singh, 1986- robust summary), indicating that parental carbon monoxide exposure to levels as low as 65 ppm alters the righting reflex of neonates, indicating damage to the developing central nervous system.
Conclusion:	NOAEL (Maternal, systemic) = 500 ppm Developmental Effects: NOAEL = 65 ppm - fetal weight LOAEL = 125 ppm - fetal weight
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to a guideline study; adequate level of detail
Key Study Sponsor Indicator:	Key

REFERENCE	
Reference:	Singh J and LH Scott. 1984. Threshold for carbon monoxide induced fetotoxicity. Teratology 30: 253-257.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	630-08-0
Test Substance:	Carbon monoxide
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:	
Type of Exposure:	Developmental toxicity study
Species:	Mouse
Other Species:	
Mammalian Strain:	CD-1
Other Strain:	

Gender:	Females
Number of Animals per Dose:	No data
Concentration:	
Dose:	0, 65, or 125, ppm
Year Study Performed :	1986
Method/Guideline Followed:	Other
GLP:	No data
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** continuous
Frequency of Treatment:	7 days/week, gestation days 7-18
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	Control group: yes, concurrent compressed air Method: This study investigated the effects of carbon monoxide exposure on pregnancy and fetal outcomes. Female albino CD-1 mice were bred overnight with males of the same strain, and the day a copulation plug was found was designated as gestation day 1. The pregnant animals were exposed continuously to 0, 65, or 125 ppm carbon monoxide in air in plexiglas environmental chambers from gestation day 7 to 18. The concentration of carbon monoxide was monitored at each chamber inlet by carbon monoxide detector. The animals were killed on gestation day 18, and the number of live pups and their weights were recorded on day 1 of birth. In order to determine the effects of prenatal CO exposure on neonatal reflex development the following were undertaken: righting feflex, negative geotaxis, aerial righting reflex. All data were analyzed by using the litter as the sampling unite. Analysis of variance followed by Sudent-Newman-Keuls test were used for comparison between control and test groups for all data. The results were based on data from 14 litters for each concentration and control.

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Female (Maternal)	=	125		ppm

LOAEL	Offspring (F1) – Fetal aerial righting reflex	=	65		ppm

Results Remarks:	No sign of maternal toxicity was observed under the conditions of exposure. However, effects were observed in the offspring. Carbon monoxide exposure did not significantly affect the mean number of live pups born/litter or their birth weight, although apparent decreases were suggestive of an effect. At 125 ppm, significant increases in the time required by the pups for righting reflexes on day 1 of birth and negative geotaxis on day 10 of birth were observed. Furthermore, these pups took three times longer for righting reflex and two times longer for negative geotaxis than the control pups. Prenatal CO exposure at both 65 and 125 ppm significantly decreased the mean aerial righting score of the pups on day 14 of birth when compared to the controls. The authors suggest that at low concentrations, prenatal carbon monoxide exposure may lead to retarded reflex development in neonates in a dose dependent manner.
Conclusion:	NOAEL (Maternal) = 125 ppm LOAEL (Developmental)= 65 ppm
RELIABILITY/DATA QUALITY	
Reliability:	Valid With Restrictions (KS=2)
Reliability Remarks:	Non guideline study; limited developmental parameters measured; adequate level of detail.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Singh J. 1986. Early Behavioral Alterations in Mice Following Prenatal Carbon Monoxide Exposure. NeuroToxicology 7: 475-482.

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	7664-41-7
Test Substance:	Ammonia
Test Substance Purity/Composition and Other Test Substance Comments:	Ammonia (NH_3) obtained by flushing of manure pits weekly.
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	
Type of Exposure:	Inhalation of ammonia in air environment
Species:	Pig
Other Species:	
Mammalian Strain:	Yorkshire x Hampshire x Chester White gilts (a young sow)
Other Strain:	
Gender:	Females only
Number of Animals per Dose:	40
Concentration:	
Dose:	7 ppm (=4.9 mg/m^3) and 35 ppm (=24.3 mg/m^3) [both values were means]
Year Study Performed :	1993
Method/Guideline Followed:	Other
GLP:	No data

Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** continuous
Frequency of Treatment:	6-weeks pre-mating; 10-weeks post-mating
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	Type: one generation Duration of test: 6 weeks prior to mating, 10 weeks post mating. Control group: none From 2-4.5 months of age, gilts were exposed naturally to Mycoplasma hyponeumoniae and Pasteurella multocida, which cause enzootic pneumonia and atrophic rhinitis, respectively. At 4.5 months of age, the gilts were moved to one of two rooms and exposed to either low (mean 7 ppm) or moderate (mean 35 ppm) aerial concentrations of ammonia. Each exposure group consisted of 40 individuals. In the room with low ammonia concentration, manure was flushed weekly to maintain a 0.3 m depth. In the room with moderate ammonia concentration, manure accumulated to 0.48 m depth. Moderate aerial ammonia concentration was obtained initially and maintained by adding anhydrous ammonia from a steel tank. Mean daily gain was determined by weighing the gilts biweekly. Half the gilts from each exposure concentration were sacrificed after 6 weeks. The remaining gilts were maintained in their respective environments, exposed daily to mature boars, bred at first estrus, and sacrificed at day 30 of gestation.

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Female (Maternal)	=	35		ppm
NOAEL	Offspring (F1)	=	35		ppm

Results Remarks:	At the end of two weeks, gilts in the moderate exposure group weighed less than those in the low exposure. After 2 weeks gilts acclimated and the mean daily gain was similar for the rest of the experiment. The gilts sacrificed at 6 weeks showed that the animals in the low exposure were heavier. At day 30 of gestation,

	number of live fetuses, fetal length, and fetus-to-corpus luteum ratio were all similar between the two groups. No statistically significant differences were noted in ovarian or uterine weights of pigs exposed to about No unexposed controls were included in the study. No statistically significant difference in fetal length was evident at 30 days of gestation in offspring of pig dams that were continuously exposed to about 7 or 35 ppm ammonia from 6 weeks before breeding until day 30 of gestation.
Conclusion:	NOAEL = 35 ppm
RELIABILITY/DATA QUALITY	
Reliability:	Not assignable (KS=4)
Reliability Remarks:	Non guideline; nonstandard species; lack of detail; no controls
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Diekman, MA., Scheidt, AB., Sutton, AL., Green, ML., Clapper, JA, Kelly, DT., and Van Alstine, WG. 1993. Growth and reproductive performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. Am J Vet Res 54(12):2128-2131.

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	7783-06-4
Test Substance:	Hydrogen Sulfide
Test Substance Purity/Composition and Other Test Substance Comments:	Hydrogen sulfide supplied by Holox gases (Cary, NC)

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/dose level)
Concentration:	
Dose:	0, 10, 30, 80 ppm
Year Study Performed :	1995
Method/Guideline Followed:	OECD 421
GLP:	Yes
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** 6 hrs/day
Frequency of Treatment:	7 days/week (females, gestation days 0-19 and postnatal days 5-18; males 70 consecutive days)
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	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, concurrent no treatment

	Method: This study investigated the effects of perinatal exposure by inhalation to hydrogen sulfide (H2S) on pregnancy outcomes, offspring prenatal and postnatal development, or offspring behavior. Virgin male and female Sprague-Dawley rats (12 rats/sex/concentration) were exposed (0, 10, 30, or 80 ppm H2S; 6h/day, 7 days/week) for 2 weeks prior to breeding. Exposures continued during a 2-week mating period (evidence of copulation = gestation day 0) and then from gestation day 0 through gestation day 19. Exposure of dams and their pups (eight rats/litter after culling) resumed between post natal day 5 and 18. Adult male rats were exposed for 70 consecutive days. Offspring were evaluated using motor activity (postnatal day 13, 17, 21, and 60 +/- 2), passive avoidance (postnatal day 22 +/- 1 and 62 +/- 3), functional observation battery (postnatal day 60 +/- 2) acoustic startle response (postnatal day 21 and 62 +/- 3) and neuropathology (postnatal day 23 +/- 2 and 61 +/- 2).

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Female (Maternal)	=	80		ppm
NOAEL	Offspring (F1)	=	80		ppm

Results Remarks:	There were no deaths and no adverse physical signs observed in F0 male or female rats during the study. A statistically significant decrease in feed consumption was observed in F0 male rats from the 80 ppm H2S exposure group during the first week of exposure. There were no statistically significant effects on the reproductive performance of the F0 rats as assessed by the number of females with live pups, litter size, average length of gestation, and the average number of implants per pregnant female. Exposure to H2S did not affect pup growth, development, or performance on any of the behavioral tests.
Conclusion:	

RELIABILITY/DATA QUALITY

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

REFERENCE	
Reference:	Dorman, DC, MF Struve, EA Gross and KA Brenneman. 2000. Fertility and developmental neurotoxicity effects of inhaled hydrogen sulfide in Sprague-Dawley rats. Neurotoxicology and Teratology. 22:71-84.

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. <u>Statistical Analysis:</u> 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.

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.

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:	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.