201-16650A

HIGH PRODUCTION VOLUME (HPV)

CHEMICAL CHALLENGE PROGRAM

TEST PLAN For:

C4-6 ISOPENTENE RICH-ETHER FRACTION STREAM

CAS No. 108083-43-8

Prepared by:

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November 14, 2007

EXECUTIVE SUMMARY

Under the U.S. Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program (Program), ExxonMobil Chemical Company committed to voluntarily compile a Screening Information Data Set (SIDS) that can be used for an initial hazard assessment of C4-6 Isopentene Rich-Ether Fraction (IRF) stream, CAS No. 108083-43-8.

Existing data and technical analyses adequately characterize the SIDS endpoints for the IRF stream and support a screening-level hazard assessment, which informs the public about the SIDS-based hazards of this substance. Sufficient data and information exist to characterize all endpoints in the HPV Program. Therefore, no additional testing is proposed.

The IRF stream is a complex substance that contains a predominant ether fraction in combination with a larger hydrocarbon fraction. A search for existing studies/information and their review identified adequate data for select constituents to characterize all SIDS endpoints for the stream. Data suggest that the IRF stream generally presents a low order of hazard for human health and low to moderate order of environmental hazard for the predominant groups of constituents as a whole. The predominant constituents of the stream are relatively volatile. Information on their fate in the environment suggests that once in the atmosphere, they will be largely degraded through physical processes at a relatively rapid rate.

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TEST PLAN FOR C4-6 ISOPENTENE RICH-ETHER FRACTION CAS No. 108083-43-8

I. INTRODUCTION

Under the U.S. Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program (Program), ExxonMobil Chemical Company committed to voluntarily compile a Screening Information Data Set (SIDS) that can be used for an initial hazard assessment of the C4-6 Isopentene Rich-Ether Fraction (IRF) stream, CAS No. 108083-43-8. Although there are no data for the stream, based on its composition, it was determined that data for three constituents can be used to characterize the SIDS endpoints because they comprise a large fraction of the stream and therefore, will define the fate and effects of the stream. The three substances are tert-amyl methyl ether (CAS No. 994-05-8), heptane (CAS No. 142-82-5), and cyclohexene (CAS No. 110-83-8).

This assessment includes data for selected physicochemical, environmental fate, and mammalian and environmental effect endpoints identified by the U.S. HPV Program. Procedures to assess the reliability of selected data for inclusion in this test plan were based on guidelines described by Klimisch *et al*. (1997) and identified within the U.S. EPA (1999) document titled *Determining the Adequacy of Existing Data*. The following sections describe the IRF stream and its manufacturing process, and data used to characterize the various endpoints in the HPV Program. After a review of the existing data, ExxonMobil Chemical Company believes that data needed to adequately assess all SIDS endpoints have been identified and that additional testing is not necessary.

II. CHEMICAL DESCRIPTION, MANUFACTURING PROCESS, AND USE

The IRF stream is composed of several constituent substances (Table 1). The substances listed in Table 1 are those found at concentrations of greater or equal to 1% in the stream. The predominant chemical fraction in this stream is the methoxypentanes, which can comprise from approximately 43 to 60% of the stream. The major methoxypentane is 2-methoxy-2-methylbutane, which is also referred to as tert-amyl methyl ether (TAME). A second chemical fraction, which can also comprise a large proportion of the stream is the heptanes. This fraction comprises approximately 18 to 24% of the stream. A third fraction, which can also comprise a significant portion of the stream, is cyclohexene, and can be as much as 6 to 9%. Together these three fractions can comprise up to 91% of the stream. All other groups or identified chemicals in Table 1 each comprise less than 5% of the stream.

In the chemical plant, a mostly C5 stream is brought into the isoamylene unit (IAU) from upstream fractionation. To remove the isoamylene (2-methyl-butene-1 and 2-methyl-butene-2), the stream is run across a catalyst that oxygenates the isoamylene into TAME (tertiary-amyl methyl ether) using methanol. This TAME is then fractionated away from the remaining C5 stream, and decomposed back to isoamylene and methanol and recovered as product. In the first methanol reaction, there are side reactions that occur that cause generation of oxygenates heavier than TAME. These

are fractionated off as the IAU heavy co-product (IRF stream). The IRF stream is then used as a feedstock to make hydrocarbon resins.

Table 1. Percent composition ranges of predominant constituents in the C4-6 Isopentene Rich-Ether Fraction stream.

C4-6 ISOPENTENE RICH-ETHER FRACTION STREAM		
Chemical Group/Chemical (~total % composition range)	**Percent Composition Range**	**Constituent***
C5 Oxygenates (~3 - 7)		
	2.5 - 2.9	2-pentanone
	2.8 - 3.9	tert-amyl alcohol
C6 Cyclic Olefin (~6 - 9)		
	5.7 - 8.5	cyclohexene
Methoxypentanes (~43 - 60)		
	2.8 - 3.3	1-methoxy-3-methylbutane
	12.7 - 15.3	2-methoxypentane
	27.7 - 40.9	2-methoxy-2-methylbutane (tert-amyl methyl ether)
Heptanes (~18 - 24)		
	1.6 - 2.4	2,3-dimethylpentane
	2.1 - 3.0	trans-1,3-dimethylcyclopentane
	2.6 - 3.7	cis-1,3-dimethylcyclopentane
	5.2 - 5.6	n-heptane
	6.4 - 9.3	2-methylhexane
Octane (~1 - 2)		
	1.2 - 1.6	2,2,4-trimethylpentane
C10 Olefins (~2)		
	2.1 - 2.3	C10 olefins (unidentified)

* IRF stream constituents at concentration ranges greater than 1%; ranges are based on results from analyses of three different streams.

III. TEST PLAN RATIONALE AND DATA SUMMARY

The predominant constituent chemical groups of the C4-6 Isopentene Rich-Ether Fraction stream include the methoxypentanes (3 constituents) at as much as 60% of the stream, the heptanes (5 constituents) at as much as 24% of the stream, and cyclohexene at as much as 9% of the stream, these combined constituent fractions, which can comprise from 67 to 91% of the stream, will be responsible for the biological effects exhibited by the stream as a whole. The few remaining chemical groups or individual chemical constituents, that are present at levels between 1% to as much as 9%, will not contribute to a greater adverse biological effect than that resulting from the two major groups. Therefore, data from a representative constituent from each of these two groups and cyclohexene will be used to characterize the overall biological and fate characteristics of the stream.

The basic strategy of this test plan for characterizing the human health hazards of the C4-6 Isopentene Rich-Ether Fraction stream is to evaluate data for the major components of the stream. The major chemical components of the stream in the C4-6 Isopentene Rich-Ether Fraction stream have been tested for human health toxicity endpoints. Available data on these components prove to provide sufficient information to develop scientific judgment-based characterizations of the human health effects of the stream for purposes of satisfying HPV program requirements. Therefore, no additional human health toxicity testing is proposed. The hazard characterization for the C4-6 Isopentene Rich-Ether Fraction stream will include the hazards of tert-amyl methyl ether (TAME), n-heptane and cyclohexene.

The environmental fate and effects of the methoxypentanes (ethers) will be characterized by 2-methoxy-2-methylbutane, also referred to as tert-amyl methyl ether (TAME), which has a SIDS dataset. Use of the TAME data to characterize the ether group in this stream is supported by calculated results from the ECOSAR computer model (ECOSAR, 2004) using EPI Suite™ (2000) modeled input data. The 48- or 96-hour data for each of the freshwater fish, daphnid, and green alga endpoints show that the three ethers are expected to cause effects within 20 mg/L of each other. The environmental fate and effects of the heptanes will be characterized by measured and calculated data for n-heptane. The environmental fate and effects of the C6 cyclic olefin component will be characterized by measured and calculated data for cyclohexene.

All TAME test data identified within this document were developed using the parent substance. Additional data for this group used to characterize the aquatic toxicity endpoints were developed using the ECOSAR computer model (ECOSAR, 2004) provided within EPI Suite™ (2000). This model applies an equation for neutral organics to estimate aquatic toxicity and is therefore considered appropriate to estimate aquatic toxicity for the representative substances.

Data used to characterize the various physicochemical, environmental fate, and environmental and mammalian health endpoints are described below.

A. Physicochemical Data

Calculated and measured TAME, heptane, and cyclohexene physicochemical data from the literature are listed in Table 2.

Table 2. Selected physico-chemical properties for three select constituents used to characterize the C4-6 Isopentene Rich-Ether Fraction stream.

ENDPOINT	TAME*	HEPTANE	CYCLOHEXENE
Melting Point (ºC)	-81.2 (U.S. EPA, 2000)	-90.6 (Lide *et al.*, 1997-1998)	-103.5 (U.S. EPA, 2000)
Boiling Point (ºC at 1012 hPa)	86.3 (Lide *et al.*, 1997-1998)	98.4 (Lide *et al.*, 1997-1998)	82.9 (U.S. EPA, 2000)
Density (g/cm^3 at 20°C)	0.770 (Lide *et al.*, 1997-1998)	0.684 (Lide *et al.*, 1997-1998)	0.81 (Verschueren, 1983)
Vapor Pressure (Pa at 25°C)	12,000 (Huttunen *et al.*, 1997)	6,133 (Daubert & Danner, 1989)	11,865 (Daubert & Danner, 1989)
Water Solubility (mg/l at 25°C)	5,468 (U.S. EPA, 2000)	3.4 (Yalkowsky & Dannenfelser, 1992)	213 (Yalkowsky & Dannenfelser, 1992)
Log K_{ow}	1.55 (20°C) (Huttunen *et al.*, 1997)	4.50 (25°C) (Sangster, 1989)	2.86 (20°C) (Hansch *et al.*, 1995)

* tert-amyl methyl ether

Conclusion

Based on data identified for TAME, n-heptane, and cyclohexene, the IRF stream will exhibit a melting range between approximately -81 to -104°C, a boiling range between approximately 83 to 98°C, a density ranging from approximately 0.8 to 0.7 g/cm^3 at 20°C, and a vapor pressure between approximately 6,133 to 12,000 Pa at 25°C. The predominant constituents of the IRF stream have water solubilities that range from 3.4 to 5,468 mg/l at 25°C and Log K_{ow} values that range from approximately 1.55 to 4.50 (Log K_{ow} values were determined at two different temperatures, 20 and 25°C).

B. Environmental Fate Data

Biodegradation

Biodegradation of an organic substance by bacteria can provide energy and carbon for microbial growth. This process results in a structural change of an organic substance and can lead to the complete degradation of that substance, producing carbon dioxide and water.

The test guideline used to assess the biodegradability of TAME was OECD 301D, Closed Bottle Biodegradation Test. This test design uses a sealed bottle, which is appropriate considering the test material is relatively volatile. The source of the microbial inoculum used in this study was a domestic wastewater treatment facility and it was not acclimated. TAME exhibited 4% biodegradation after 28 days (Bealing, 1995).

The test guideline used to assess the biodegradability of n-heptane was the standard method for the examination of water and waste water (APHA, 1971). The source of the microbial inoculum used in this study was a silt loam soil and it was not acclimated. The average biodegradation based on theoretical biological oxygen demand for n-heptane on days 2, 5, 10, and 20 was 28, 63, 70, and 70%, respectively (Haines and Alexander, 1974).

Biodegradability of cyclohexene was determined following test guidelines of the Japanese Ministry of International Trade and Industry (MITI). The study design is comparable to OECD 301C (Modified MITI Test). The concentration of cyclohexene in the study was 100 mg/l, with a concentration of 30 mg/l of inoculum. The source of the inoculum was activated sludge and it was not acclimated. Cyclohexene exhibited 0% biodegradation after 28 days, based on BOD (CITI, 1992).

Conclusion

Based on data for TAME and n-heptane, the IRF stream is expected to demonstrate an overall low extent of biodegradation. The ether fraction of the stream, which is the predominant fraction, is not expected to demonstrate significant biodegradation because the constituents that comprise this fraction will biodegrade to a similar extent as was exhibited by TAME. The cyclohexene fraction is also not expected to biodegrade to any appreciable extent. In comparison, the hydrocarbon fraction is expected to demonstrate a higher extent of biodegradability, in particular, the paraffinic fraction as a whole is expected to exhibit a moderate extent of biodegradation with heptane as the potentially most rapidly biodegradable. However, in the environment, the fate of the IRF stream constituents have the potential to partition primarily to air because the they have relatively high vapor pressures, which suggests that they can volatilize to the air at a rapid rate if released.

Photodegradation – Photolysis

Direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance in aqueous solution. If the absorbed energy is high enough, then the resultant excited state of the chemical may undergo a transformation. A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer (Harris, 1982a).

An approach to assessing the potential for a substance to undergo photochemical degradation is to assume that degradation will occur in proportion to the amount of light wavelengths >290 nm absorbed by constituent molecules (Zepp and Cline, 1977). The oxygen non-bonding electrons in ethers do not give rise to absorption above 160 nm, which is why pure ether solvents can be used in spectroscopic studies. Consequently,

the ether fraction of the IRF stream is not subject to photolytic processes in the aqueous environment.

Similarly, saturated and unsaturated hydrocarbons like those in the IRF stream do not absorb light above 290 nm. Therefore, the hydrocarbon constituents of this stream will not exhibit photolytic degradation.

Conclusion

Based on the potential for photolysis of ethers and hydrocarbons, this process is not expected to significantly contribute to the degradation of constituents of the IRF stream.

Photodegradation – Atmospheric Oxidation

Photodegradation can be measured (US EPA, 1999a) or estimated using an atmospheric oxidation potential (AOP) model accepted by the EPA (US EPA, 1999b). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation.

The constituents of the IRF stream have the potential to volatilize to air, based on the vapor pressure of three of the predominant constituents, where they are subject to atmospheric oxidation. In air, IRF stream constituents can react with photosensitized oxygen in the form of hydroxyl radicals ($^{\bullet}OH$). The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows) (U.S. EPA, 2000) calculates a chemical half-life for a 12-hour day (the 12-hour day half-life value normalizes degradation to a standard day light period during which hydroxyl radicals needed for degradation are generated), based on an $^{\bullet}OH$ reaction rate constant and a defined $^{\bullet}OH$ concentration.

TAME has a calculated half-life in air of 24.6 hours or 2.1 days (12-hour day), based on a rate constant of 5.22 x 10^{-12} cm^3/molecule*sec and an $^{\bullet}OH$ concentration of 1.5 x 10^6 $^{\bullet}OH$ /cm^3. Heptane has a calculated half-life in air of 18.7 hours or 1.6 days (12-hour day), based on a rate constant of 6.87 x 10^{-12} cm^3/molecule*sec and an $^{\bullet}OH$ concentration of 1.5 x 10^6 $^{\bullet}OH$ /cm^3. In comparison, cyclohexene has a calculated half-life in air of 2.1 hours or 0.17 days (12-hour day), based on a rate constant of 61.52 x 10^{-12} cm^3/molecule*sec and an $^{\bullet}OH$ concentration of 1.5 x 10^6 $^{\bullet}OH$ /cm^3.

Conclusion

Atmospheric oxidation via hydroxyl radical attack can be a significant route of degradation for constituents in the IRF stream. Based on calculated values for three chemicals that are representative of the majority of stream constituents, IRF stream constituents are expected to have an atmospheric half-life of approximately 2 days or less as a result of indirect photolysis by hydroxyl radical attack.

Stability in Water (Hydrolysis)

Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals with leaving groups that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Neely, 1985). The lack of a suitable leaving group renders a compound resistant to hydrolysis. Ether and hydrocarbon constituents of the IRF stream are resistant to hydrolysis because they lack functional groups that are

hydrolytically reactive and Harris (1982b) identifies ether groups as generally resistant to hydrolysis.

Conclusion

Hydrolysis will not contribute to the removal from the environment of constituents in the IRF stream**.**

Chemical Distribution In The Environment (Fugacity Modeling)

Fugacity-based multimedia modeling provides basic information on the relative distribution of a chemical between selected environmental compartments (i.e., air, soil, water, sediment, suspended sediment, and biota). Two widely used fugacity models are the EQC (Equilibrium Criterion) Level I and Level III model (Mackay, 1998a; Mackay, 1998b).

The input data required to run a Level I model include basic physicochemical parameters; distribution is calculated as percent of chemical partitioned to 6 compartments (air, soil, water, suspended sediment, sediment, biota) within a unit world. Level I data are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical may to partition, based on selected physical parameters. The Level III model uses the same physical parameters as the Level I model, but also requires half-life degradation data for the air, soil, water, and sediment compartments, as well as emission parameters for the air, water, and soil compartments.

Results of the Mackay Level I and Level III environmental distribution models for three representative stream constituents are shown in Tables 3 and 4, respectively.

Table 3. Environmental distribution as calculated by the Mackay (1998a) Level I fugacity model for select constituents used to characterize the C4-6 Isopentene Rich-Ether Fraction stream.

ENVIRONMENTAL COMPARTMENT	TAME DISTRIBUTION* (%)	HEPTANE DISTRIBUTION** (%)	CYCLOHEXENE DISTRIBUTION† (%)
Air	97.77	99.91	99.82
Water	2.16	<0.01	0.11
Soil	0.07	0.08	0.07
Sediment	<0.01	<0.01	<0.01
Suspended Sediment	<0.01	<0.01	<0.01
Biota	<0.01	<0.01	<0.01

* Distribution is based on the following model input parameters for TAME (tert-amyl methyl ether):

Molecular Weight	102.18
Temperature	25° C
Log K_{ow}	1.55
Water Solubility	5,468 g/m^3
Vapor Pressure	12,000 Pa
Melting Point	-81.22° C

** Distribution is based on the following model input parameters for heptane:

Molecular Weight	100.21
Temperature	25° C
Log K_{ow}	4.50
Water Solubility	3.4 g/m^3
Vapor Pressure	6,133 Pa
Melting Point	-90.6° C

† Distribution is based on the following model input parameters for cyclohexene:

Molecular Weight	82.15
Temperature	25° C
Log K_{ow}	2.86
Water Solubility	213 g/m^3
Vapor Pressure	11,865 Pa
Melting Point	-103.5° C

Table 4. Environmental distribution as calculated by the Mackay (1998b) Level III fugacity model for select constituents used to characterize the C4-6 Isopentene Rich-Ether Fraction stream.

ENVIRONMENTAL COMPARTMENT	TAME DISTRIBUTION* (%)	HEPTANE DISTRIBUTION** (%)	CYCLOHEXENE DISTRIBUTION† (%)
Air	26.2	26.0	3.0
Water	55.2	48.5	78.5
Soil	18.6	13.9	17.3
Sediment	0.1	11.6	1.2

* Distribution for TAME (tert-amyl methyl ether) is based on the following model input parameters, reaction half-life in hours as predicted using EPI SuiteTM (2000), and a model default emission rate of 1000 kg/hr into each of the air, water, and soil compartments:

Molecular Weight	102.18	Reaction half-life (hr):	
Temperature	25° C	Air (gaseous)	46.7
Log K_{ow}	1.55	Water (no susp. part.)	360
Water Solubility	5,468 g/m^3	Bulk Soil	720
Vapor Pressure	12,000 Pa	Bulk Sediment	3,240
Melting Point	-81.22° C		

** Distribution for heptane is based on the following model input parameters, reaction half-life in hours as predicted using EPI SuiteTM (2000), and a model default emission rate of 1000 kg/hr into each of the air, water, and soil compartments:

Molecular Weight	100.21	Reaction half-life (hr):	
Temperature	25° C	Air (gaseous)	35.9
Log K_{ow}	4.50	Water (no susp. part.)	208
Water Solubility	3.4 g/m^3	Bulk Soil	416
Vapor Pressure	6,133 Pa	Bulk Sediment	1,870
Melting Point	-90.6° C		

† Distribution for cyclohexene is based on the following model input parameters, reaction half-life in hours as predicted using EPI SuiteTM (2000), and a model default emission rate of 1000 kg/hr into each of the air, water, and soil compartments:

Molecular Weight	82.15	Reaction half-life (hr):	

Temperature	25° C	Air (gaseous)	2.09
Log K_{ow}	2.86	Water (no susp. part.)	360
Water Solubility	213 g/m^3	Bulk Soil	720
Vapor Pressure	11,865 Pa	Bulk Sediment	7,200
Melting Point	-103.5° C		

Conclusion

Results of the Mackay Level I model suggest that the predominant constituents of the IRF stream will partition primarily to the air, >97%. These results are largely explained by their vapor pressures. In comparison, the Level III model suggests that the majority of the IRF stream will partition to the water compartment, approximately 49 to 78%, followed by the air compartment at approximately 3 to 26%, and soil compartment at approximately 14 to 19%. These results are explained by the model parameters, but in particular the default emission rates and degradation half-lives.

C. Aquatic Toxicity Data

Data are available to characterize the potential freshwater fish acute, invertebrate acute, and freshwater alga toxicity of the IRF stream, based on data for three constituents, TAME, n-heptane, and cyclohexene (Tables 5 through 7). TAME demonstrated a measured 96-hour trout (*Oncorhynchus mykiss*) LC_{50} toxicity value of 580 mg/L (API, 1995a) and a measured 48-hour invertebrate (*Daphnia magna*) EC_{50} toxicity value of 100 mg/L (API, 1994). The lowest green alga (*Selenastrum capricornutum*) 72-hour EC_{50} toxicity value was for biomass and measured 230 mg/L (Fortum, 2003). The 72-hour NOEC value from this study was 77 mg/L.

The measured TAME data were compared with data calculated (Table 5) by the ECOSAR model (2004). This model is considered appropriate to estimate the aquatic toxicity for this class of chemicals. The calculated data compared favorably with the measured data. The calculated freshwater fish acute, invertebrate acute, and freshwater alga toxicity values ranged between 127 to 208 mg/L.

Table 5. Measured and calculated aquatic toxicity values for TAME (tert-amyl methyl ether).

ENDPOINT	MEASURED VALUE (mg/L)	CALCULATED VALUE* (mg/L)
Fish 96-hr LC_{50}	580 (API, 1995a)	201
Daphnid 48-hr EC_{50}	100 (API, 1994)	208
Alga 72-hr EbC_{50}	230 (Fortum, 2003)	na
Alga 96-hr EC_{50}	na	127
Alga 72-hr NOEC	77 (Fortum, 2003)	na
Alga 96-hr ChV**	na	10**

na - not available

* Model input parameters for ECOSAR (2004):

Log K_{ow}	1.55
Water Solubility	5,468 g/m^3
Melting Point	-81.2° C

** ChV (chronic) value

Measured n-heptane data are available for a freshwater invertebrate and two marine invertebrate species (Table 6). Heptane demonstrated a 48-hour invertebrate (*Daphnia magna*) EC_{50} toxicity value of 1.5 mg/L (TNO, 1986). Two marine invertebrate species, *Chaetogammarus marinus* and *Mysidopsis bahia*, demonstrated 96-hour LC_{50} toxicity values of 0.2 and 0.1 mg/L, respectively (TNO, 1986).

The measured n-heptane data were compared with data calculated (Table 6) by the ECOSAR model (2004). This model is considered appropriate to estimate the aquatic toxicity for this class of chemicals. The calculated data compared favorably with the measured data. The calculated freshwater fish acute, invertebrate acute, and freshwater alga toxicity values ranged between 0.3 to 0.4 mg/L.

Table 6. Measured and calculated aquatic toxicity values for n-heptane.

ENDPOINT	MEASURED VALUE (mg/L)	CALCULATED VALUE* (mg/L)
Fish 96-hr LC_{50}	na	0.33
Daphnid 48-hr LC_{50}	1.5 (TNO, 1986)	0.42
Alga 96-hr EC_{50}	na	0.31
Alga 96-hr ChV**	na	0.13
Marine Invert. 96-hr LC_{50}	0.2 (TNO, 1986)	na
Marine Invert. 96-hr LC_{50}	0.1 (TNO, 1986)	na

na - not available

* Model input parameters for ECOSAR (2004):
Log K_{ow} 4.50
Water Solubility 3.4 g/m^3
Melting Point -90.6° C

** ChV - chronic value

Measured cyclohexene data are available for a freshwater fish (Table 7). Cyclohexene demonstrated a 96-hour medaka (*Oryzias latipes*) LC_{50} toxicity value of >10 mg/L (CITI, 1992). An additional study with Coho Salmon (*Oncorhynchus kisutch*) reported no significant mortalities up to 100 ppm (Morrow *et al.*, 1975) for cyclohexene in artificial seawater. However, the study was performed in open vessels, and therefore the data was considered questionable.

The measured cyclohexene data were compared with data calculated (Table 7) by the ECOSAR model (2004). This model is considered appropriate to estimate the aquatic toxicity for this class of chemicals. The calculated data compared favorably with the measured data. The calculated freshwater fish acute, invertebrate acute, and freshwater alga toxicity values ranged between 5.8 to 8.7 mg/L.

Table 7. Measured and calculated aquatic toxicity values for cyclohexene.

ENDPOINT	MEASURED VALUE (mg/L)	CALCULATED VALUE* (mg/L)
Fish 96-hr LC_{50}	>10	7.6
Daphnid 48-hr LC_{50}	na	8.7
Alga 96-hr EC_{50}	na	5.8
Alga 96-hr ChV**	na	1.0

na - not available

* Model input parameters for ECOSAR (2004):
Log K_{ow} 2.86
Water Solubility 213 g/m^3
Melting Point -103.5° C

** ChV - chronic value

Conclusion

The predominant constituent chemical groups of the C4-6 Isopentene Rich-Ether Fraction stream include the methoxypentanes (3 constituents), which when combined can range from approximately 43 to 60% of the stream, the heptanes (5 constituents), which when combined can range from approximately 18 to 24% of the stream, and cyclohexene, which can range from 6 to 9% of the stream. These combined constituent fractions, which can comprise up to 91% of the stream, will be responsible for the biological effects exhibited by the stream as a whole. Although the methoxypentanes (represented by toxicity data for TAME) comprise the larger percentage of the stream, they demonstrate much lower toxicity in comparison to the heptanes (represented by toxicity data for n-heptane). Cyclohexene also demonstrates much lower toxicity in comparison to n-heptane. The heptanes are contained by the IRF stream in sufficient concentration to exert effects on fish, invertebrates, and algae at levels demonstrated by n-heptane. Therefore, the effect range characterized by the data for n-heptane represents the potential aquatic toxicity of the IRF stream, which can range from 0.1 to 1.5 mg/L.

D. Mammalian Toxicity Data

Acute Toxicity

Data are available to characterize the potential acute toxicity of the C4-6 Isopentene Rich-Ether Fraction stream, based on data for three constituents, Tertiary amyl methyl ether (TAME), cyclohexene and n-heptane. The oral rat LD_{50} values for TAME and cyclohexene were approximately 2100 mg/kg (Daughtrey and Bird, 1995) and 1000-2000 mg/kg (OECD, 2002), respectively. The dermal LD_{50} values for TAME and cyclohexene were >3160 mg/kg (ExxonMobil, 1985a) and >20 mL/kg (OECD, 2002), respectively. The inhalation rat LC_{50} values for TAME and n-heptane were >5.4 mg/L (Amoco, 1991a) and >29 mg/L (HEDSET, 1982), respectively. Four-hour inhalation exposure of rats to 21388 mg/m^3 (6370 ppm) cyclohexene produced no deaths (OECD, 2002).

In summary, available acute toxicity data on predominant constituents of the C4-6 Isopentene Rich-Ether Fraction stream demonstrated a low order of acute oral, dermal, and inhalation toxicity. No further testing is proposed.

Genotoxicity

In vitro

Three constituents of the C4-6 Isopentene Rich-Ether Fraction stream have been evaluated in several *in vitro* genotoxicity assays. TAME, cyclohexene and n-heptane were negative in a bacterial reverse gene mutation assay (Ames test) in *Salmonella typhimurium* and/or *Escherichia coli* with and without S-9 metabolic activation (Brooks et al., 1982; Daughtrey and Bird, 1995; OECD, 2002). Cyclohexene and n-heptane showed no evidence of genotoxic activity in the mammalian chromosomal aberration assays (Brooks et al., 1982; OECD, 2002). n-Heptane was also negative in a mitotic gene conversion assay using *Saccharomyces cerevisiae* JD1 (Brooks et al., 1982).

TAME was tested in an *in vitro* Mammalian Chromosomal Aberration Test (American Petroleum Institute, 1997b). In this study, TAME was tested in cultured Chinese hamster ovary (CHO) cells for induction of chromosomal aberrations, both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and five doses of TAME. The doses tested were 313, 625, 1250, 2500, and 5000 μg/ml. In the absence of S9, a statistically significant increase in aberrant cells was observed at 2500 and 5000 ug/ml, and a dose response was observed. In the presence of S9, a statistically significant increase in aberrant cells was observed at all concentrations, and a dose response was observed. In conclusion, based on these results, TAME was clastogenic under the conditions of this assay.

In vivo

TAME was evaluated *in vivo* for its ability to induce micronuclei in bone marrow polychromatic erythrocytes (PCEs) in CD-1 mice (Daughtrey and Bird, 1995). TAME was diluted in corn oil and administered as a single intraperitoneal injection at doses of 0.15, 0.375, and 0.75 g/kg. Cyclophosphamide was dissolved in water and used as the positive control at a dose of 40 mg/kg. Animals from the appropriate groups were euthanized by CO_2 at approximately 24, 48 and 72 hours after administration of test article. Animals dosed with cyclophosphamide were taken at 24 hours only. Each group consisted of 10 animals (5/sex/group) per time point. At death, both femurs from each animal were removed and bone marrow was recovered and suspended in fetal bovine serum. Bone marrow slides were prepared and stained with acridine orange prior to microscopic evaluation. One thousand polychromatic erythrocytes from each animal were examined for micronuclei formation. In addition, the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) was determined by counting 1000 erythrocytes (PCEs and NCEs). No increase in micrucucleus frequency was observed at any dose level of TAME or at any of the bone marrow collection times. The positive control produced statistically significant increases in micronucleus frequencies in both males and females. Overt marrow toxicity, as measured by a statistically significant decrease in the percentage of polychromatic erythrocytes, was not observed in any of the groups dosed with TAME. The percentages of polychromatic erythrocytes observed were within the normal range. Thus, these data indicated that

TAME did not cause clastogenic effects in mouse bone marrow.

In summary, *in vitro* genotoxicity testing of cyclohexene and n-heptane demonstrated no evidence of genotoxicity. TAME was not mutagenic in an *in vitro* Ames assay but was found to be clastogenic in an *in vitro* chromosome aberration study. However, as no evidence of genotoxicity was observed in an *in vivo* mouse micronucleus test, the weight of evidence suggests that TAME is not a mutagen. Based on these data on predominant constituents, no additional testing on the C4-6 Isopentene Rich-Ether Fraction stream is proposed.

Repeated Dose Toxicity

A number of repeated dose toxicity studies have been conducted on TAME, cyclohexene and n-heptane.

A 28-day repeated dose inhalation toxicity study was conducted with TAME vapor in Sprague-Dawley rats (Amoco, 1991a; White *et al.*, 1995). In this study, the rats (14/sex/group) were exposed to TAME vapor at target concentrations of 0, 500, 2000, and 4000 ppm for 6 hours per day, 5 days per week for 4 weeks. Three out of 14 males and 4 out of 14 females in the 4000 ppm group died during the study, three animals during the first week, two during the second week and two during the third week. The 2000 ppm and 4000 ppm groups showed signs of central nervous system depression as well as other signs of toxicity, e.g., lacrimation, dyspnea, rales, diarrhea, piloerection, etc. Significant decreases in body weight gain were observed in the 4000 ppm males resulting in significantly reduced mean body weights during weeks 2 - 4. No other significant effects on body weight were reported. Evaluation of gross pathology revealed that absolute brain weights were significantly decreased in the 4000 ppm males and that absolute liver weights were significantly increased in the 2000 ppm males and 4000 ppm females. Many relative organ weights were increased for the 4000 ppm males due to the reduced body weights of these animals. No treatment-related histopathological findings were noted. TAME produced minimal effects on clinical chemistry and hematology parameters. A No Observed Adverse Effect Level (NOAEL) of 500 ppm was determined in this study.

A 28-day repeated oral dose toxicity study was conducted with TAME in Sprague-Dawley rats (Daughtrey and Bird, 1995). In this study, the rats (5/sex/group) were dosed with 0, 125, 500, and 1000 mg/kg TAME in corn oil by gavage at a dose volume of 2 ml/kg. Vehicle control animals received corn oil only. The dosing regimen was once daily, 7 days a week, for a period of 29 days.

Four animals (two male, two female) in the high-dose group died during the course of the study. Of these four, two deaths were attributed to dosing accidents. The remaining two deaths were presumed to be test material-related, although a precise cause of death could not be identified. All other animals survived to the scheduled termination. Overall, in-life observations were unremarkable. Lung rales and anogenital staining of the fur were observed at a low frequency in the high-dose group. The majority of animals of either sex did not exhibit any unusual symptoms or behaviors. Mean body weights of high-dose males were significantly lower than those of control males at day 7, day 21, and day 28. Mean body weight gain in high-dose females was also lower than in control females, although the difference was not statistically significant. Food consumption in high-dose males and females was also

significantly reduced compared to controls during week 1. During week 2, food consumption was significantly reduced only in high dose males. A dose-related increase in adrenal weights was observed that was statistically significant in the mid- and high-dose males. A similar increase in adrenal weights was not observed in female rats dosed with TAME. Relative kidney weights were also increased in mid- and high-dose male rats compared to control.

Hematology and serum chemistry values were generally similar across dose groups. Activated partial thromboplastin time was statistically increased in the high-dose male (but not female) group. However, this small increase was not believed to be biologically meaningful. The mean serum glucose value was also significantly reduced in the high-dose male group. The biological significance of this finding was unknown, however a similar decrease in serum glucose was not observed in high-dose females. No treatment-related tissue lesions were observed during the histopathological examination. Any changes observed were limited to naturally occurring lesions that were present in approximately equal frequency in all groups, including controls. Of note, the organ weight increases observed in the kidney and adrenals were not accompanied by any histopathological changes. The NOAEL in this study was determined to be 500 mg/kg/day.

In a 13-week repeated dose toxicity study conducted by the American Petroleum Institute (1997a), F344 rats (51/sex - control and high dose; 41/sex - low and mid-dose) and CD-1 mice (46/sex -control and high dose; two groups each; 36/sex -low and mid-dose) were exposed by whole body inhalation to TAME at target concentrations of 0, 240, 1500, and 3500 ppm for 6 hours/day, 5 day/week for 13 weeks (minimum 65 exposures). A new high dose group of mice at 2500 ppm and corresponding control group were established due to high mortality at 3500 ppm. The results for rats and mice are presented separately.

In rats, a number of effects were observed at the highest dose used, 3500 ppm. These effects included a low incidence of mortality (2/102), abnormal clinical signs (lethargy and prostration), acute neurological effects, decreased body weight and body weight gain, effects on hematology (increased platelet counts), effects on clinical chemistry (increases in total protein, albumin and globulin), and a number of effects on organ weights. The effects on the kidneys of the male rats were consistent with the male rat specific α2u-globulin syndrome and were not considered to be relevant to risk assessment in humans. Exposure of rats at 1500 ppm resulted in effects including post exposure clinical signs, acute neurological effects (males only), increased platelet count in males, increases in total protein, albumin and globulin and effects on liver and kidney (only in females) weight. An increase in liver weights of male rats exposed to 250 ppm was also observed. Many of these resolved after the 4 week recovery period. On histopathological examinations, no dose-related changes were observed in the liver. No test material-related changes in motor activity were observed at any doses. The NOAEL for rats was 1500 ppm in this study.

In this study, high mortality was observed in mice exposed to 3500 and 3000 ppm. A number of effects were observed at the highest dose used in the main study, 2500 ppm. These included 27 deaths among 92 mice, post-exposure clinical signs, effects on a number of clinical chemistry parameters, and increased liver weights. Exposure of mice at 1500 ppm resulted in effects including post exposure clinical signs, increased globulin

in males at week 6 and effects on liver weights in males. Many of these resolved after the 4 week recovery period. Liver cell proliferation studies showed increases in the labelling index of hepatocytes and centrilobular hepatocellular hypertrophy was observed in the 2500, 1500 and 250 ppm animals. Centrilobular hepatocellular hypertrophy is considered an adaptive response to increased metabolic load. The NOAEL for mice was determined to be 1500 ppm.

Effects of repeated exposure to cyclohexene were evaluated as part of an OECD 422, combined repeated toxicity study with reproductive/developmental toxicity screen in SD rats (OECD, 2002). Twelve male and twelve female rats received gavage doses of 0 (corn oil), 50, 150 and 500 mg/kg/day cyclohexene. Males were dosed for 48 days and females for 42-53 days from 14 days before mating to day 4 of lactation throughout the mating and pregnancy period. Salivation was observed in 3 of 12 males and 2 of 12 females at 150 mg/kg/day and in all of 12 males and 12 females at 500 mg/kg/day. This sign was observed only for about 5 minutes after dosing at 150 mg/kg/day but up to 6 hours after dosing at 500 mg/kg/day. No significant changes of body weight, food consumption and hematological findings for both sexes and urinalysis findings for males were detected. Blood chemical examination showed a decrease in triglyceride in males at 150 and 500 mg/kg/day, and increases in total bilirubin in males at 500 mg/kg/day and in total bile acid in females at 50 mg/kg/day and in both sexes at 150 mg/kg/day and above. In males of the 500 mg/kg/day group, there was an increase in relative kidney weight. On histopathological examinations, no dose-related changes were observed. The increase in total bile acid observed in females at 50 mg/kg/day was not considered to be an adverse effect because of no accompanying changes. Therefore, based on salivation at 150 mg/kg/day and above, the NOAEL for repeated dose toxicity was considered to be 50 mg/kg/day for both sexes.

A 26-week inhalation toxicity study with n-heptane was conducted in Sprague-Dawley rats (API, 1980). In this study, the rats (15/sex/group) were exposed by inhalation to 0, 398 and 2970 ppm n-heptane for 6 hours/day, 5 day/week for 26 weeks. There were no treatment-related deaths during the study. The only treatment-related observations were labored breathing or rapid breathing and slight prostration during the first week of study during exposure only, and anogenital fur staining and dry rales during weekly observations. No significant changes of body weight, hematology or urinalysis for both sexes were detected. Serum alkaline phosphatase was significantly elevated in female high dose rats and slightly elevated in low dose females. All other clinical chemistry values appeared normal with the exception of one male in the high dose group whose serum glutamic pyruvic transaminase and serum alkaline phosphatase levels were markedly elevated when compared to all other male rats on test. Proteinuria, elevated specific gravity and ketones were observed but do not appear to be related to treatment. The effects observed are consistent with acute central nervous system (CNS) depression and generally abated by the second week of the study. Under the conditions of this study, the Low Observed Adverse Effect Level (LOAEL) for acute CNS depression was 2970 ppm and the NOAEL for systemic toxicity was 2970 ppm.

In a 30-week inhalation neurotoxicity study, Sprague-Dawley rats (6-9 males/dose group) were exposed by inhalation to air or 1500 ppm n-heptane for 9 hours/day, 5 days/week for 30 weeks (Frontali et al., 1981). The primary objective of this study was to assess the appearance of polyneuropathy and urinary metabolites in rats following exposure to analytical grade solvents frequently used in Italian shoe factories. Nerve

tissue was examined microscopically. None of the animals developed signs of neuropathy. There were no differences in weight gain of rats exposed to n-heptane compared to controls. Differences between mean values for hindlimb spreads observed in treated animals and controls were not statistically significant. No histological signs of giant oxonal degeneration were noted in rats treated at 1500 ppm. Under the condition of this test, the NOAEL for repeated dose toxicity was considered to be 1500 ppm.

In summary, data are available to adequately characterize the repeated dose toxicity of C4-6 Isopentene Rich-Ether Fraction (IRF) stream. The IRF stream is expected to have a low order of repeated dose toxicity. No further testing is proposed.

Reproductive and Developmental Toxicity

Predominant constituents of the C4-6 Isopentene Rich-Ether Fraction stream have been evaluated for reproductive and developmental toxicity.

A two-generation reproductive toxicity study of inhaled TAME vapor was conducted in Sprague-Dawley rats (Tyl *et al.*, 2003). In this study, weanling F0 rats (30/sex/group) inhaled TAME vapor at 0, 250, 1500, or 3000 ppm 5 day/week and 6 h/day for 10 weeks, with vaginal cytology evaluated for weeks 8-10. The F0 animals then produced F1 offspring, with exposure 7 days a week from mating through to lactation. During the F1 prebreed exposure period, vaginal patency, preputial separation (PPS) and vaginal cytology were evaluated. The F1 animals were mated, with F2 anogenital distance measured on postnatal day zero. At F2 weaning 30 of each gender per group were selected for postwean retention, with no exposures, through vaginal patency and PPS. Body weights, feed consumption and clinical signs were recorded throughout the study. Adult F0 and F1 systemic toxicity was present at 1500 and 3000 ppm. Minor adult male reproductive toxicity was present at 3000 ppm. There were no adult effects on vaginal cyclicity, estrous cycle length, mating, fertility, pregnancy, gestational length or ovarian and uterine weights. There were no treatment-related gross or histopathologic findings in parental male or female systemic or reproductive organs. Offspring toxicity was present at 1500 and 3000 ppm. The NOAEL for adult reproductive toxicity was 1500 ppm for males and 3000 ppm for females. The NOAEL for offspring toxicity was 250 ppm in rats under the conditions of this study.

A developmental toxicity study was conducted by TAME vapor inhalation exposure in two pregnant rodent species (Welsch *et al.*, 2003). Timed-pregnant Sprague-Dawley rats and CD-1 mice, 25 animals per group, inhaled TAME vapors containing 0, 250, 1500, or 3500 ppm for 6 hours a day on gestational days (gd) 6-16 (mice) or 6-19 (rats). The developmental toxicity hazard potential was evaluated following the study design draft guidelines and end points proposed by the United States Environmental Protection Agency.

In the present study, inhalation of TAME by pregnant rats from gestational days 6-19 resulted in manifestations of maternal toxicity at 1500 and 3500 ppm. These effects were expressed by reductions in body weight (at 3500 ppm only), feed consumption and weight gain, and TAME exposure-induced clinical signs of toxicity. There was no evidence of maternal toxicity at 250 ppm. The increased maternal relative liver weight at 3500 ppm that occurred when maternal body weight was actually reduced may be due to induction of metabolizing enzymes and a concurrent increase in liver mass. There was a clear indication of maternal accommodation to the highest TAME exposure

concentration, as evidenced by diminution in incidence and intensity of clinical signs such as ataxia, lethargy and slow respiration over time. Developmental toxicity occurred only at 3500 ppm and was expressed as reduced fetal body weights per litter. There was no evidence of treatment-related teratogenicity at any of the three exposure concentrations and no other developmental effects. Almost all of the fetal malformation and variation findings were those commonly observed in historical control Sprague-Dawley rat fetuses and in published control databases. Therefore, the NOAEL was 250 ppm for maternal toxicity and 1500 ppm for developmental toxicity in rats under the conditions of this study.

In mice, the inhalation of TAME vapors during gd 6-16 resulted in maternal toxicity at 3500 ppm, including maternal mortality (4/25), reductions in body weight, weight gain and treatment-related clinical signs of toxicity. At 1500 ppm, mice exhibited reduced feed consumption (only for gd 6-9) and limited treatment-related clinical signs of toxicity. There was no evidence for maternal toxicity at 250 ppm. The increased maternal absolute and relative liver weights at 1500 and 3500 ppm may have been due to induction of metabolizing enzymes and therefore increase in tissue mass. There was also a clear indication of reduced pharmacological effects with time and maternal accommodation to the top two exposure concentrations. This interpretation was supported by observations of mortality at 3500 ppm early in the exposure period (gd 6-9) only and diminution over time in the incidence of clinical signs of toxicity, such as ataxia, lethargy, gasping and slow respiration. The increased relative liver weight may have been due, at least in part, to the reduced body weights of the dams at termination at 3500 ppm. Developmental toxicity was present at 3500 ppm, expressed specifically as increased incidence of late fetal deaths, reduced fetal body weights per litter and increased incidences of cleft palate (an external malformation) and of enlarged lateral ventricles of the cerebrum (a visceral variation). At 1500 ppm, three fetuses in three litters also exhibited cleft palate (with none observed at 250 or 0 ppm). This increase was not statistically significant, but it is considered biologically relevant and related to maternal TAME exposure. The finding of one additional litter at 1500 ppm with three multiply malformed fetuses (out of nine live fetuses total) may be unrelated to treatment because these malformations were not observed at 3500 ppm and were limited to only one litter at 1500 ppm. The observation of cleft palate in fetuses at 1500 and 3500 ppm appears to be consistent with a proposed mechanism for cleft palate in mice exposed to methyl tertiary butyl ether (MTBE). Maternal exposure to MTBE with anesthetic qualities at high concentrations associated with maternal stress results in elevated endogenous corticosteroid levels, which cause cleft palate to the developing offspring in mice (Bevan *et al.*, 1997). Although those hormone levels were not determined in the present study, the biological mode of action of TAME appears to be similar and comparable to that of MTBE, as judged by clinical observations. At high exposure concentrations in mice, TAME exerts depressant effects on the central nervous system that resemble anesthetic properties and are preceded by a pronounced excitatory stage. Therefore, the brain stimulation and excitation may have induced a rise in endogenous corticosteroid levels in the mouse dams. The occurrence of a significantly increased incidence of fetal cleft palate at the 3500 ppm exposure level, coincident with maternal toxicity, suggests that stress of the dams is a contributing factor. Mice are sensitive to stress, and cleft palate occurs in offspring if the pregnant dams experience stress such as food and water deprivation, transportation, restraint or low humidity. That corticosteroids cause cleft palate in susceptible mouse strains is well documented.

The increased incidence of enlarged lateral ventricles of the fetal cerebrum at 3500 ppm is consistent with developmental delay because the fetuses at this exposure concentration exhibited mean body weights per litter of about 60% of the concurrent control group values. There were no notable developmental effects at 250 ppm. Therefore, the NOAEL for maternal and developmental toxicity in mice was 250 ppm in the present study.

In an OECD 422, combined repeated toxicity study with reproductive/developmental toxicity screen, cyclohexene was administered to SD(Crj:CD)IGS rats by gavage at doses of 0, 50, 150 and 500 mg/kg/day for 48 days from 14 days prior to mating in males and for 42-53 days from 14 days prior to mating to day 4 of lactation throughout the mating and pregnancy period in females (OECD, 2002). Regarding the reproductive ability of parent animals, no effects were detected on the estrus cycle, copulation index, fertility index, gestation length, numbers of corpora lutea and implantations, implantation index, gestation index, delivery index, parturition or maternal behavior. Regarding the developmental parameters, no effects were detected on viability, body weight, general appearance or autopsy findings of offspring. The NOAEL for reproduction/developmental toxicity was considered to be 500 mg/kg/day.

The available data on predominant constituents of the C4-6 Isopentene Rich-Ether Fraction (IRF) stream prove adequate to support a screening level assessment of the reproductive and developmental toxicity of the IRF stream. Furthermore, these data indicate that the IRF stream is expected to have a low order of reproductive and developmental toxicity.

Conclusion

Mammalian toxicology data on three constituents of the IRF stream, TAME, n-heptane and cyclohexene, have shown a low order of acute toxicity by the oral, dermal and inhalation routes of exposure. Repeated exposure to these constituents is not expected to produce target organ toxicity nor cause harm to reproduction or the developing fetus. There is no evidence of causing adverse effects on genetic material. The available data compiled for predominant constituents prove adequate to support a screening level hazard assessment of the IRF stream. Therefore, no additional human health toxicity testing is proposed.

Table 8. Mammalian toxicity endpoint summary for TAME.

TOXICITY ENDPOINT		RESULTS	REFERENCE
Acute	Inhalation	LC50 >5.4 mg/L	Amoco, 1991a
	Oral	LD50 = ~2100 mg/kg	Daughtrey and Bird, 1995
	Dermal	LD50 >3160 mg/kg	ExxonMobil, 1985a
Irritation	Skin	Minimal irritant	Amoco, 1991b
	Eye	Minimal irritant	ExxonMobil, 1985b
Sensitization		Not a dermal sensitizer	American Petroleum Institute, 1995b
Repeated Dose		Rat: NOAEL = 1500 ppm Mouse: NOAEL = 1500 ppm	American Petroleum Institute, 1997a
Reproductive		NOAEL for adult reproductive toxicity = 1500 ppm (males), >3000 ppm (females) NOAEL for offspring toxicity = 250 ppm	Tyl *et al.*, 2003
Developmental		NOAEL for maternal toxicity = 250 ppm (rat, mouse) NOAEL developmental toxicity = 1500 ppm (rat), 250 ppm (mouse)	Welsch, 2003
Neurotoxicity		Acute CNS depression were only observed at high doses immediately after exposure. All effects were completely reversible within 24 hours.	American Petroleum Institute, 1997a
Genotoxicity	*In vitro* Ames *Salmonella* assay	Negative	Daughtrey and Bird, 1995
	In vitro chromosome aberration	Positive - TAME was clastogenic under the conditions of this assay	American Petroleum Institute, 1997b
	In vivo micronucleus	Negative - TAME was not clastogenic to mouse bone marrow	Daughtrey and Bird, 1995

Table 9. Mammalian toxicity endpoint summary for Cyclohexene.

TOXICITY ENDPOINT		RESULTS	REFERENCE
Acute	Inhalation	LC50 >21388 mg/m^3	OECD, 2002
	Oral	LD50 = 1000-2000 mg/kg	
	Dermal	LD50 >16220 mg/kg	
Irritation	Skin		
	Eye		
Sensitization			
Repeated Dose		NOAEL = 50 mg/kg/day (rat)	
Reproductive		NOAEL = 500 mg/kg/day (rat)	
Developmental		NOAEL = 500 mg/kg/day (rat)	
Neurotoxicity			
Genotoxicity	*In vitro* Ames *Salmonella* assay	Negative	
	In vitro chromosome aberration	Negative	
	In vivo micronucleus		

Table 10. Mammalian toxicity endpoint summary for n-Heptane.

TOXICITY ENDPOINT		RESULTS	REFERENCE
Acute	Inhalation	LC50 >29.29 mg/L	HEDSET, 1982
	Oral		
	Dermal		
Irritation	Skin		
	Eye		
Sensitization			
Repeated Dose		NOAEL = 2970 ppm (rat)	American Petroleum Institute, 1980
Reproductive			
Developmental			
Neurotoxicity		No signs of neuropathy and no histological evidence of giant axonal degeneration were noted in rats.	Frontali et al., 1981
Genotoxicity	*In vitro* Ames *Salmonella* assay	Negative	Brooks et al., 1982
	In vitro chromosome aberration	Negative	Brooks et al., 1982
	In vivo micronucleus		

IV. TEST PLAN SUMMARY

A search for existing studies/information identified adequate data to characterize all endpoints under the U.S. EPA HPV Program using data from representative constituents of the predominant fractions in the IRF stream. The three constituents were TAME, n-heptane and cyclohexene. Adequate data for TAME, n-heptane and cyclohexene are shown in Table 11.

Table 11. TAME, cyclohexene, and n-heptane data availability and adequacy for endpoints in the HPV Program.

	Mammalian Toxicity						**Environmental Toxicity**			**Environmental Fate**				**Physical/Chemical Properties**					
	Acute Tox.	Genetic Pt. Mut.	Genetic Chrom.	Repeat Dose	Devel.	Repro.	Acute Fish	Acute Invert.	Alga Tox.	Photo-deg.	Hydrol.	Fug.	Biodeg.	Melt. Pt.	Boil. Pt.	Dens.	Vap. Pres.	Water Sol.	K_{ow}
TAME	**A**	**A**	**A**	**A**	**A**	**A**	**A/C**	**A/C**	**C**	**T**	**T**	**C**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
Cyclohexene	**A**	**A**	**A**	**A**	**A**	**A**	**C**	**C**	**A/C**	**T**	**T**	**C**	**-**	**A**	**A**	**A**	**A**	**A**	**A**
Hep-tane	**A**	**A**	**A**	**A**	**-**	**-**	**C**	**A/C**	**C**	**T**	**T**	**C**	**A**	**A**	**A**	**A**	**A**	**A**	**A**

A Adequate measured data available
C Adequate computer model data available
T Adequate technical discussion available

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