201-16840A

HIGH PRODUCTION VOLUME (HPV)

CHEMICAL CHALLENGE PROGRAM

TEST PLAN For:

GASES, PETROLEUM, EXTRACTIVE, C4-6 ISOPENTENE RICH REACTION PRODUCTS WITH METHANOL, ETHER FRACTION, HYDROGENATED, CRACKED, ISOPENTENE FRACTION

CAS No. 108083-44-9

Prepared by:

ExxonMobil Chemical Company

June 23, 2010

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 Gases, Petroleum, Extractive, C4-6 Isopentene Rich Reaction Products with Methanol, Ether Fraction, Hydrogenated, Cracked, Isopentene Fraction (C4-6 IRRP Fraction), CAS No. 108083-44-9.

Existing data and technical analyses adequately characterize the SIDS endpoints for the C4-6 IRRP Fraction 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 C4-6 IRRP Fraction 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 C4-6 IRRP Fraction stream generally presents a low order of hazard for human health and a 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.

TABLE OF CONTENTS

TEST PLAN FOR C4-6 IRRP FRACTION
CAS No. 108083-44-9

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 Gases, Petroleum, Extractive, C4-6 Isopentene Rich Reaction Products with Methanol, Ether Fraction, Hydrogenated, Cracked, Isopentene Fraction (C4-6 IRRP Fraction) stream, CAS No. 108083-44-9. 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 n-hexane (CAS No. 110-54-3), 2,4-dimethylpentane (CAS No. 108-08-7), and cyclohexane (CAS No. 110-82-7).

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 C4-6 IRRP Fraction 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 C4-6 IRRP Fraction stream is composed of several constituent substances (Table 1). The predominant chemical fractions in this stream are C5 to C8 aliphatic hydrocarbons, which can comprise approximately 90% of the stream. The remaining 10% is made up of olefins (<1%), methoxypentanes or ethers (6%), furans (<1%) and aromatic hydrocarbons (<2%).

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 light co-product stream. The IAU light co-product stream is then sent back to the refinery for further processing.

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

C4-6 IRRP Fraction Stream		
Chemical Group	**Constituent**	**Percent Composition**
C5 Aliphatics		
	pentanes	0.6
C6 Aliphatics		
	hexanes	24.5
	hexane	0.1
	cyclohexane	20.8
C7 Aliphatics		
	2,4-dimethylpentane	35.4
	3-methylhexane	0.2
	heptanes	3.1
	heptane	0.1
	dimethylcyclopentane	1.3
	methylcyclohexane	0.3
C8 Aliphatics		
	octanes +	3.6
C6 Olefins		
	2-methyl-1-pentene	0.1
	trans-3-hexene	0.4
	2,3-dimethyl-2-butene	0.3
Methoxypentanes		
	TAME	3.7
	SAME	1.5
Aromatics		
	benzene	1.8
Furans		
	2-methylfuran	1.0

III. TEST PLAN RATIONALE AND DATA SUMMARY

The predominant constituent chemical groups of the C4-6 IRRP Fraction stream include C6 to C7 Aliphatics (9 constituents) at as much as 80% of the stream, these combined constituent fractions, 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 4%, will not contribute to a greater adverse biological effect than that resulting from the two major groups. Therefore, data from representative constituents from each of these two groups 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 IRRP Fraction stream is to evaluate data for the major components of the stream. The major chemical components of the stream in the C4-6 IRRP 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 IRRP Fraction stream will include the hazards of n-hexane, 2,4-dimethylpentane and cyclohexane.

The environmental fate and effects of the C4-6 IRRP Fraction stream will be characterized by n-hexane, 2,4-dimethylpentane, and cyclohexane. A SIDS dataset exists for cyclohexane. Use of the constituent data to characterize the environmental fate and effects for this stream is supported by calculated results from the ECOSAR computer model (ECOSAR, 2004) using EPI Suite™ (2000) modeled input data. The acute aquatic toxicity data for each of the freshwater fish, daphnid, and green alga endpoints show that the representative constituent fractions are expected to cause effects within a relatively narrow range from 0.2 to 4.5 mg/l.

Additional data for this stream 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. No additional environmental fate and effects testing is proposed.

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

A. Physicochemical Data

Calculated and measured n-hexane, 2,4-dimethylpentane, and cyclohexane 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 IRRP Fraction stream.

ENDPOINT	Hexane	2,4-Dimethylpentane	Cyclohexane
Melting Point (ºC)	-95.3 (Lide, 2003)	-119.5 (Lide, 2003)	6.6 (Lide, 2003)
Boiling Point (ºC at 1012 hPa)	68.7 (Lide, 2003)	80.4 (Lide, 2003)	80.7 (Lide, 2003)
Density (g/cm^3 at 20°C)	0.659 (Riddick *et al.*, 1986)	0.668 (Riddick *et al.*, 1986)	0.774 (Riddick *et al.*, 1986)
Vapor Pressure (Pa at 25°C)	20,131 (Boublik *et al.*, 1984)	10,586 (Daubert & Danner, 1989)	12,919 (Chao *et al.*, 1983)
Water Solubility (mg/l at 25°C)	9.5 (McAuliffe, 1966)	5.5 (Yalkowsky & Dannenfelser, 1992)	55.0 (McAuliffe, 1966)
Log K_{ow}	3.90 (20°C) (Hansch *et al.*, 1995)	3.63 (25°C) (U.S. EPA, 2000)	3.44 (20°C) (Hansch *et al.*, 1995)

Below are the physico-chemical properties for four minor constituent chemicals that can be found in the C4-6 IRRP Fraction stream at levels between 2 and 4%, and which can vary between production runs. These values were reported in various peer-reviewed journals and can be found on the TOXNET website (http://www.toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?index.html).

TAME : MP = -81.2°C;, BP = 86.3°C; D = 0.770; VP = 12,000 Pa; WS = 5468 mg/l; log Kow = 1.55

heptane : MP = -90.6°C;, BP = 98.4°C; D = 0.684; VP = 6,133 Pa; WS = 3.4 mg/l; log Kow = 4.5

octane : MP = -56.8°C;, BP = 125.7°C; D = 0.699; VP = 1,880 Pa; WS = 0.66 mg/l; log Kow = 5.2

benzene : MP = 5.5°C;, BP = 80.1°C; D = 0.879; VP = 12,639 Pa; WS = 1.79E3 mg/l; log Kow = 2.13

Conclusion

Based on data identified for n-hexane, 2,4-dimethylpentane, and cyclohexane, the C4-6 IRRP Fraction stream will exhibit a melting range between approximately 6.6 to -119°C, a boiling range between approximately 68 to 81°C, a density ranging from approximately 0.6 to 0.8 g/cm^3 at 20°C, and a vapor pressure between approximately

10,500 to 20,100 Pa at 25°C. The predominant constituents of the IRF stream have water solubilities that range from 5.5 to 55 mg/l at 25°C and Log K_{ow} values that range from approximately 3.44 to 3.90.

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.

A test procedure used to develop data for substances in this stream was OECD 301F Guideline (Manometric Respirometry Test), which uses a continuously stirred, closed system, measuring oxygen consumption. This method is recommended when assessing the biodegradability of poorly water soluble, volatile constituents like those in this stream. The microbial inocula originated from a domestic wastewater treatment facility and were not acclimated for the test procedures.

Data for heptane was developed following this method and is available to be used as read-across for n-hexane. Heptane was evaluated in triplicate test systems at a concentration of approximately 45 mg/l, and exhibited 74% biodegradation after 28 days, based on ThOD (ExxonMobil, 1996). Linear and methyl branched C_6 and C_7 hydrocarbon isomers are expected to biodegrade to similar extents because of their similarity in structure and the conservative nature of microbial metabolic processes. In data developed using the Japanese MITI test, for example, n-hexane, at a concentration of 100 mg/l, achieved 100% of its theoretical BOD in 4 weeks using an activated sludge inoculum at 30 mg/l (CITI, 1992).

Additional data for n-heptane was also developed following standard methods 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 cyclohexane was determined following OECD 301F test guidelines. Cyclohexane was evaluated in triplicate test systems at a concentration of approximately 34 mg/l, and exhibited 77% biodegradation after 28 days, based on ThOD (ExxonMobil, 1995). A lag-phase of about 12 days was observed and the 10-day criteria was fulfilled. The first step of cyclohexane biodegradation is oxidation to cyclohexanol (Verschueren, 1983). Cyclohexanol can clearly be considered as readily biodegradable (CITI, 1992). Overall, it can be concluded that cyclohexane is readily biodegradable in the aquatic environment.

The biodegradation potential of 2,4-dimethylpentane was assessed using data comparing freshwater experimental biodegradation half-lives with computer model predictions. Primary biodegradation half-life predictions were made using the BioHCWin module of the EPISuite v4.0 model, and the results include values for n-hexane, n-heptane, and 2,4-dimethylpentane of 4.7, 5.5, and 5.6 days, respectively. In experiments with gasoline and biodiesel hydrocarbons, un-acclimated, freshwater aerobic biodegradation half-lives were reported by Prince, et.al. (2007, 2008) for n-

hexane, n-heptane, and 2,4-dimethylpentane. Prince, et.al., reported half-life values of 6.5, 4.5, and 9.1 days for these three constituent chemicals, respectively. According to the authors, biodegradation of hydrocarbons followed a semi-sequential process with n-alkanes degrading first, followed by iso-alkanes. It was also noted that smaller (C5 and lower) n-alkanes and iso-alkanes degraded slower than their larger (C7 to C12) counterparts. Degradation of the iso-alkanes favored the 2-methyl form, followed by the 3- and 4-methyl forms, and that the degradation of monomethylalkanes began before the biodegradation of the n-alkanes was complete. Biodegradation of the di-methyl hydrocarbons lagged behind the mono-methyl compounds, but were eventually consumed during the study.

Based on these results, 2,4-dimethylpentane is not expected to persist in the aquatic environment.

The ether fraction of the stream, which is small (approximately 5%), is not expected to demonstrate significant biodegradation.

Conclusion

Based on experimental data for n-hexane, n-heptane, 2,4-dimethylpentane and cyclohexane, as well as QSAR predictions, the C4-6 IRRP Fraction stream is expected to demonstrate an overall high extent of biodegradation. However, in the environment, the fate of the C4-6 IRRP Fraction stream constituents have the potential to partition primarily to air because 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). Saturated and unsaturated hydrocarbons like those in the C4-6 IRRP Fraction stream do not absorb light above 290 nm. Therefore, the hydrocarbon constituents of this stream will not exhibit photolytic degradation.

Similarly, 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 C4-6 IRRP Fraction stream is also not subject to photolytic processes in the aqueous environment.

Conclusion

Based on the potential for photolysis of hydrocarbons and ethers, this process is not expected to significantly contribute to the degradation of constituents of the C4-6 IRRP

Fraction 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 C4-6 IRRP Fraction 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, C4-6 IRRP Fraction stream constituents can react with photosensitized oxygen in the form of hydroxyl radicals (•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 •OH reaction rate constant and a defined •OH concentration.

n-Hexane has a calculated half-life in air of 23.4 hours or 1.96 days (12-hour day), based on a rate constant of 5.46 x 10^{-12} cm^3/molecule*sec and an •OH concentration of 1.5 x 10^6 •OH /cm^3. 2,4-dimethylpentane has a calculated half-life in air of 18.7 hours or 1.6 days (12-hour day), based on a rate constant of 6.85 x 10^{-12} cm^3/molecule*sec and an •OH concentration of 1.5 x 10^6 •OH /cm^3. Cyclohexane has a calculated half-life in air of 15.1 hours or 1.3 days (12-hour day), based on a rate constant of 8.48 x 10^{-12} cm^3/molecule*sec and an •OH concentration of 1.5 x 10^6 •OH /cm^3. Atkinson (1989) reported experimental •OH rate constants for hexane, 2,4-dimethylpentane, and cyclohexane of 5.61 x 10^{-12} cm^3/molecule*sec, 5.16 x 10^{-12} cm^3/molecule*sec, and 7.49 x 10^{-12} cm^3/molecule*sec, respectively.

Conclusion

Atmospheric oxidation via hydroxyl radical attack can be a significant route of degradation for constituents in the C4-6 IRRP Fraction stream. Based on calculated and experimental values for the three chemicals that are representative of the majority of stream constituents, C4-6 IRRP Fraction 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. Hydrocarbon and ether constituents of the C4-6 IRRP Fraction 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 C4-6 IRRP Fraction 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 IRRP Fraction stream.

ENVIRONMENTAL COMPARTMENT	HEXANE DISTRIBUTION* (%)	2,4-DIMETHYLPENTANE DISTRIBUTION** (%)	CYCLOHEXANE DISTRIBUTION† (%)
Air	99.97	99.98	99.91
Water	0.02	<0.01	0.03
Soil	0.01	0.01	0.06
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 n-Hexane:

Molecular Weight	86.18
Temperature	25° C
Log K_{ow}	3.90
Water Solubility	9.5 g/m^3
Vapor Pressure	20,131 Pa
Melting Point	-95.3° C

** Distribution is based on the following model input parameters for 2,4-dimethylpentane:

Molecular Weight	100.21
Temperature	25° C
Log K_{ow}	3.63
Water Solubility	5.5 g/m^3
Vapor Pressure	10,586 Pa
Melting Point	-119.5° C

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

Molecular Weight	84.16
Temperature	25° C
Log K_{ow}	3.44
Water Solubility	55.0 g/m^3
Vapor Pressure	12,919 Pa
Melting Point	6.6° C

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

ENVIRONMENTAL COMPARTMENT	HEXANE DISTRIBUTION* (%)	2,4-DIMETHYLPENTANE DISTRIBUTION** (%)	CYCLOHEXANE DISTRIBUTION† (%)
Air	21.3	20.0	15.8
Water	63.3	70.6	67.3
Soil	4.3	2.8	12.9
Sediment	11.1	6.6	4.0

* Distribution for n-hexane 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	86.18	Reaction half-life (hr):	
Temperature	25° C	Air (gaseous)	23.5
Log K_{ow}	3.90	Water (no susp. part.)	360
Water Solubility	9.5 g/m^3	Bulk Soil	720
Vapor Pressure	20,131 Pa	Bulk Sediment	7,200
Melting Point	-95.3° C		

** Distribution for 2,4-dimethylpentane 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)	18.7
Log K_{ow}	3.63	Water (no susp. part.)	360
Water Solubility	5.5 g/m^3	Bulk Soil	720
Vapor Pressure	10,586 Pa	Bulk Sediment	7,200
Melting Point	-119.5° C		

† Distribution for cyclohexane 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	84.16	Reaction half-life (hr):	
Temperature	25° C	Air (gaseous)	15.1
Log K_{ow}	3.44	Water (no susp. part.)	360
Water Solubility	55.0 g/m^3	Bulk Soil	720
Vapor Pressure	12,919 Pa	Bulk Sediment	7,200
Melting Point	6.6° C		

Conclusion

Results of the Mackay Level I model suggest that the predominant constituents of the C4-6 IRRP Fraction stream will partition primarily to the air, >99%. These results are largely explained by their vapor pressures. In comparison, the Level III model suggests that the majority of the C4-6 IRRP Fraction stream will partition to the water compartment, approximately 63 to 71%, followed by the air compartment at

approximately 15 to 21%, and soil and sediment compartments at approximately 3 to 13%. 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 C4-6 IRRP Fraction stream, based on data for three constituents, n-hexane, 2,4-dimethylpentane, and cyclohexane (Tables 5 through 7). n-Hexane demonstrated a measured 48-hour invertebrate (*Daphnia magna*) EC_{50} toxicity value of 2.1 mg/l (TNO, 1986). A 24-hour LC_{50} toxicity value for goldfish (*Carassius auratus*) of 4 mg/l was also reported by Verschueren (1983) using a modified ASTM D1345 test guideline (Shell Chemie, 1975). Two marine invertebrate species, *Chaetogammarus marinus* and *Mysidopsis bahia*, demonstrated 96-hour LC_{50} toxicity values of 0.4 mg/l (TNO, 1986).

The measured n-hexane 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 0.9 to 1.3 mg/l.

Table 5. Measured and calculated aquatic toxicity values for n-Hexane

ENDPOINT	MEASURED VALUE (mg/l)	CALCULATED VALUE* (mg/l)
Fish 96-hr LC_{50}	4 (Verschueren, 1983)	1.0
Daphnid 48-hr EC_{50}	2.1 (TNO, 1986)	1.3
Alga 72-hr EbC_{50}	na	na
Alga 96-hr EC_{50}	na	0.9
Alga 72-hr NOEC	na	na
Alga 96-hr ChV**	na	0.3**
Marine Invert. 96-hr LC_{50}	0.4 (TNO, 1986)	0.1

na - not available

* Model input parameters for ECOSAR (2004):

Log K_{ow}	3.90
Water Solubility	9.5 g/m^3
Melting Point	-95.3° C

** ChV (chronic) value

Measured test data was not available for 2,4-dimethylpentane. Measured data for a suitable analog, n-heptane, was used to compare to modeled 2,4-dimethylpentane data. are available for a freshwater invertebrate and two marine invertebrate species (Table 6). n-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 estimated for 2,4-dimethylpentane (Table 6) by the ECOSAR model (2004). As stated previously, 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 0.2 to 2.6 mg/l.

Table 6. Measured and calculated aquatic toxicity values for 2,4-Dimethylpentane.

ENDPOINT	MEASURED VALUE (mg/l)	CALCULATED VALUE* (mg/l)
Fish 96-hr LC_{50}	na	2.2
Daphnid 48-hr LC_{50}	1.5 (TNO, 1986)	2.6
Alga 96-hr EC_{50}	na	2.0
Alga 96-hr ChV**	na	0.5
Marine Invert. 96-hr LC_{50}	0.2 (TNO, 1986)	0.2

na - not available

* Model input parameters for ECOSAR (2004):
Log K_{ow} 3.63
Water Solubility 5.5 g/m^3
Melting Point -119.5° C

** ChV - chronic value

Measured cyclohexane data are available for a freshwater fish (Table 7). Cyclohexane demonstrated a 96-hour fathead minnow (*Pimephales promelas*) LC_{50} toxicity value of 4.53 mg/l (Geiger, *et.al*., 1987). The study was performed under flow through conditions and the results are based on measured concentrations. An additional study with Rainbow Trout (*Oncorhynchus mykiss*) reported a 96-hour LL_{50} toxicity value of 3.2 mg/l (EMBSI, 2001a). The study was performed under static, no-headspace conditions, with test solutions renewed at 24-hour intervals. Results are based on nominal concentrations of the test substance. Cyclohexane demonstrated a 48-hour invertebrate (*Daphnia magna*) EC_{50} toxicity value of 0.9 mg/l (TNO, 1986). The lowest green alga (*Selenastrum capricornutum*) 72-hour EC_{50} toxicity value was for biomass and measured 3.4 mg/l (EMBSI, 1998). Two marine invertebrate species, *Chaetogammarus marinus* and *Mysidopsis bahia*, demonstrated 96-hour LC_{50} toxicity values of 2.2 mg/l (TNO, 1986). Many other results for cyclohexane are available in the open literature, but most studies were performed in static open systems without analytical monitoring. Due to the high volatility of cyclohexane, they therefore cannot be considered as valid and are not reported here.

The measured cyclohexane 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 2.2 to 3.3 mg/l.

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

ENDPOINT	MEASURED VALUE (mg/l)	CALCULATED VALUE* (mg/l)
Fish 96-hr LC_{50}	4.53 (Geiger, *et.al*, 1987)	2.8
Daphnid 48-hr LC_{50}	0.9 (TNO, 1986)	3.3
Alga 96-hr EC_{50}	3.4 (EMBSI, 1998)	2.2
Alga 96-hr ChV**	na	0.5
Marine Invert. 96-hr LC_{50}	2.2 (TNO, 1986)	0.3

na - not available

* Model input parameters for ECOSAR (2004):
Log K_{ow} 3.44
Water Solubility 55.0 g/m^3
Melting Point 6.6° C

** ChV - chronic value

Conclusion

The predominant constituent chemical groups of the C4-6 IRRP Fraction stream include C6 Aliphatics (3 constituents), which when combined can comprise approximately 45% of the stream, and C7 Aliphatics (6 constituents), which when combined can comprise approximately 40% of the stream. These combined constituent fractions, which can comprise up to 85% of the stream, will be responsible for the biological effects exhibited by the stream as a whole. The aquatic toxicity data for constituents from this stream are expected to fall within a relatively narrow range regardless of the isomer or mixture of isomers, because the constituent chemicals are neutral organic hydrocarbons whose toxic mode of action is non-polar narcosis (Ramos *et. al*., 1998). The toxic mechanism of short-term toxicity for these chemicals is disruption of biological membrane function (Van Wezel and Opperhuizen, 1995), and the differences between toxicities (i.e., LC/LL_{50}, EC/EL_{50}) can be explained by the differences between the target tissue-partitioning behavior of individual constituent chemicals (Verbruggen *et. al*., 2000).

The existing fish toxicity database for hydrophobic, neutral organic chemicals, which compose the constituents of this stream, supports a critical body residue (CBR) for these chemicals between approximately 2-8 mmol/kg fish (wet weight) (McCarty *et al*., 1991; McCarty and Mackay, 1993). The CBR is the internal concentration of a toxicant that causes mortality. When normalized to lipid content for most organisms, the CBR is approximately 50 µmol/g of lipid (Di Toro *et al*., 2000). Therefore, only hydrocarbon substances with components of sufficient water solubility, such that their molar sum in solution is high enough to produce a total partitioning to the organism of approximately 50 µmol of hydrocarbon per gram of lipid will demonstrate lethality. The C4-6 IRRP Fraction stream is expected to exhibit acute aquatic toxicity in the range from 0.2 to 4.5 mg/l.

D. Mammalian Toxicity Data

Acute Toxicity

Data are available to characterize the potential acute toxicity of the C4-6 IRRP Fraction stream, based on data for cyclohexane, n-hexane, n-heptane, and iso-octane suitable analogs of 2,4-dimethylpentane. Rat oral LD_{50} values of > 5,000 mg/kg (Phillips Petroleum Company, 1982a), 29,800 mg/kg (Deichmann and Le Blanc, 1943) and 8,000-39,000 mg/kg (Kimura et al., 1971) have been reported for cyclohexane. The oral rat LD50 values for hexane are 28,720 mg/kg, or 15,840 and 29,700 mg/kg for juvenile and adult rats (HSDB, 2005). The dermal LD_{50} value for cyclohexane was >2000 mg/kg (Phillips Petroleum Company, 1982c). The inhalation rat LC_{50} values (4hr) for cyclohexane, n-hexane and n-heptane were >32.88 mg/L (Phillips Petroleum Company, 1982b), 48,000 ppm, and >29 mg/L (HEDSET, 1982), respectively. Swiss mice (n=4) were exposed to 1,000, 2,000, 4,000, 8,000, 16,000, 32,000, 64,000 or 128,000 ppm of iso-octane for 5 minutes. Iso-octane was highly irritating even at the lower concentrations. At 32,000 ppm, all of the mice stopped breathing within 4 min.. Considerable sensory and motor irritation and irregular respiration were noted with no apparent anesthesia (Swann *et al*., 1974). A 1 hour acute inhalation exposure of rabbits to cyclohexane vapor produced effects on the central nervous system, with exposure-related observations including convulsions, tremors, hyperactivity, rapid respiration, cyanosis and diarrhea; all the animals exposed to 89.6 mg/L died (Treon *et al*., 1943).

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

Genotoxicity

In vitro

Two constituents of the C4-6 IRRP Fraction stream and n-heptane and 2,2,4-trimethylpentane as analogs for 2,4-dimethylpentane have been evaluated in several *in vitro* genotoxicity assays. Cyclohexane, n-hexane 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 (Mortlemans *et al*., 1986; NTP, 1991; Brooks *et al*., 1982). n-Hexane and n-heptane showed no evidence of genotoxic activity in the mammalian chromosomal aberration assays (Daughtrey *et al*., 1994; NTP, 1991; Brooks *et al*., 1982). n-Heptane was also negative in a mitotic gene conversion assay using *Saccharomyces cerevisiae* JD1 (Brooks *et al*., 1982).

Cyclohexane and n-hexane were negative for forward mutations in the mouse lymphoma L5178Y tk+/- assay (API, 1986; Phillips Petroleum Company, 1982g; Hazleton Laboratories, 1992). Similarly, cyclohexane was negative in an *in vitro* sister chromatid exchange test while n-hexane showed a marginally increased incidence of sister chromatid exchanges in chinese hamster ovary cells in the presence of S9 (not dose dependent) (Phillips Petroleum Company, 1982h; NTP, 1991). n-Hexane induced polyploidy in Chinese hamster lung fibroblast cells (Ishidate *et al.*, 1984). There were no detected increases in gene mutations at the thymidine kinase (TK) locus or in sister chromatid exchanges (SCEs) when a human lymphoblastoid cell line, TK6, was treated with a saturated (5% v/v) solution of 2,2,4-trimethylpentane in cell culture medium for 3 hours in the presence and absence of rat liver S9 fraction (Richardson et al., 1986).

Cyclohexane was also tested in the unscheduled DNA synthesis test at doses of 10^{-2} – 10^{-3} and 10^{-4} M in DMSO (Perocco *et al*., 1983). Human lymphocytes (+ or – S9 mix) were cultured for 4 hours in the presence or absence of cyclohexane. The effects on the DNA synthesis were measured through cellular [^{3}H]TdR uptake. Cyclohexane induced a marked inhibition of [^{3}H]TdR uptake in the S9 mix-lacking cultures while the corresponding cellular viabilities were unaffected. No effect was seen with metabolic activation. The effects seen without metabolic activation were not dose-dependent; solvent controls and negative controls were highly variable. Decrease of the uptake for the highest dose was within the values of the controls. No conclusion was drawn from this study. DNA synthesis was inhibited in human lymphocytes in the presence of concentrations of n-hexane from 10^{-4} – 10^{-2} M but only at cytotoxic concentrations (Perocco *et al*., 1983). Unscheduled DNA synthesis (UDS) was not induced in isolated male F344 hepatocytes exposed to 2,2,4-trimethylpentane at final media concentrations of 0.33, 1.00, or 3.33% (high dose may be cytotoxic) (Loury et al., 1986).

Cyclohexane was found to be negative when tested alone in the DNA cell binding assay in the groups treated with liver extract or with lysozyme and liver extract. A positive finding (1.6% only) was found in the group treated with cyclohexane + lysozyme at the highest dose (100 uM). This result is considered doubtful because it is a very slight increase and also because this effect is not found in the group minus cyclohexane + lysozyme + liver extract (Kubinsky *et al.*, 1981).

In vivo

In vivo cyclohexane has been studied in a rodent bone marrow cytogenetic assay (American Petroleum Institute, 1982). Groups of 10 male and female Sprague Dawley rats were exposed by inhalation to atmospheres of 0, 97, 307 and 1,042 ppm for 6 hours per day for 5 days (350-1,050 -3,650 mg/m3). Samples of bone marrow cells were taken for cytogenetic analysis 6 hours after completion of the final dose. A positive control, triethyleneamine, showed a significant increase in structural aberration frequency. For cyclohexane a small but statistically significant increase in numerical aberrations was recorded in low and medium dose females, and pooled data at the low dose groups of both sexes. There was no information on general toxicity; no decrease on mitotic index was seen at all the doses tested. However, the authors of the report concluded that the lack of a dose-related response indicated that these increases were not of biological importance. Moreover, the numerical aberrations parameter had often shown great variation in this laboratory, having no statistical significance even for positive controls (numerical data is not available). It can be considered that cyclohexane does not produce chromosomal aberrations under the conditions of this test.

Test for the genotoxic potential of n-hexane *in vivo* have been predominantly negative. No dominant lethal mutations were induced following n-hexane exposure in CD-1 mice (Mast et al., 1988b; Litton Bionetics, 1980). Also n-hexane did not induce chromosomal aberrations and micronuclei in bone marrow cells of B6C3F1 mice injected intraperitoneally (Shelby and Witt, 1995). A slight, but significant, increase in the number of chromosomal mutations induced by n-hexane in albino rat bone marrow cells

has been reported (Hazleton Laboratories, 1992). In addition, an *in vivo* bone marrow cytogenetic assay found that male albino rats exposed to 150, 300, and 600 ppm of n-hexane for 5 days experienced a significant increase in chromosomal aberrations at all treatment levels compared with controls (Hazleton Laboratories, 1992). No increase in the incidence of sister chromatid exchanges in *in vivo* mouse bone marrow cells was seen with intraperitoneal doses of 500, 1,000, or 2,000 mg/kg n-hexane. (NTP, 1991). The dosed groups displayed slight increases in chromosomal aberrations, but this increase was not considered to be significant.

McLaren et al. (1994) investigated the induction of DNA double-strand breaks and poly-ADP-ribosylation in the renal cortex of male Wistar rats administered 12 mmol/kg (~1370 mg/kg) of 2,2,4-trimethylpentane via gavage for 5 consecutive days. Treatment failed to induce poly-ADP-ribosylation or a significant increase in DNA double-strand breaks in the renal cortex.

In summary, the weight of the evidence for *in vitro* genotoxicity testing of cyclohexane, n-hexane, n-heptane, and 2,2,4-trimethylpentane and *in vivo* genotoxicity testing of cyclohexane, n-hexane, and 2,2,4-trimethylpentane indicates no strong evidence for genotoxicity. Based on these data on predominant constituents, no additional testing on the C4-6 IRRP Fraction stream is proposed.

Repeated Dose Toxicity

A number of repeated dose toxicity studies have been conducted on cyclohexane, n-hexane, and n-heptane, 2-methylpentane, 3-methylpentane and 2,2,4-trimethylpentane, as suitable analogs for 2,4-dimethylpentane.

Cyclohexane

A two-week repeated dose inhalation range finding study was conducted with Crl CD.BR rats exposed (whole body) to cyclohexane vapor (Haskell Laboratory, 1995). In this study, the rats were exposed to cyclohexane vapor at target concentrations of 0, 3,000, 6,000, and 9,000 ppm. Nine exposures, each lasting 6 hours were performed in total. A slight but significant decrease in body weight gain was observed in males treated with the high dose (9,000 ppm). Only a minimal increase in mitotic index figures in the hepatocytes of males at 6,000 ppm and higher and in females at 9,000 ppm was detected; no other treatment related findings were observed for systemic toxicity. In particular, no modification in absolute and relative liver weights was noted in these studies. Based on these findings, a No Observable Adverse Effect Level (NOAEL) of 3,000 ppm was determined for systemic toxicity. For neurotoxic effects, diminished responses to stimulus were observed from day 2 at 9,000 ppm and from 7 exposures at 6,000 ppm. No effect was observed in a Functional Observational Battery (FOB). A NOAEL of 3,000 ppm was reported for neurotoxic effects in rats. This study served as a range-finding study for a 90 day inhalation toxicity study.

In a 13-week repeated dose toxicity study conducted by Haskell Laboratory, 1996a (Malley *et al*., 2000), CD BR rats (20/sex/group for controls and high concentration and 10/sex/group for intermediary concentration groups) were exposed by whole body inhalation to cyclohexane at target concentrations of 0, 500, 2,000, and 7,000 ppm for 6 hours/day, 5 day/week for 13 weeks (66 exposures). Ten rats per month were allowed a one-month recovery period for control group and 7,000 ppm groups. After 45 and 90

days of exposure, blood and urine were collected for evaluation of clinical pathology parameters. Gross pathology, organ weight, macroscopic and microscopic examinations were performed at the end of the study.

No treatment-related effects were observed on body weight, body weight gain, food consumption, urine analysis and clinical examinations. A slight decrease (not significant) in succinate dehydrogense and lactate dehydrogenase was observed in males and females at 7,000 and 2,000 ppm at both sampling times. In males exposed to 7,000 ppm a slight increase in adrenals weight was observed at the end of the recovery period. This finding was not observed at the end of the 90 day exposure so the relevance and significance is questionable. In the 7,000 ppm group, a statistically significant increase in the relative liver weight with hepatic hypertrophy was observed in males (10/10), concurrent with an increase in the incidence of centrilobular hypertrophy in both sexes (9/10 males and 5/10 females). This finding was partially reversible in the one-month recovery period. For neurological effects, decreases in or absences of response to auditory stimulations were observed with a dose-response relationship from 500 ppm. In the 500 ppm group, there was a decrease in response on treatment days 61, 66, 67 and 68. In the 2,000 ppm group, there was decrease in the response during 16 exposures and no response during 50 exposures. In the 7,000 ppm group, a decreased response was observed in one exposure and no response was observed in the other 65 exposures. These effects were transient, and as no clinical observations of compromised neurological function were detected they were considered to be due to a reversible sedation caused by cyclohexane. The NOAEL for neurological effects was 500 ppm while the NOAEL for hepatic effects was 2,000 ppm. However, the partially reversible hepatic effects observed in males at 7,000 ppm were slight and may be considered of an adaptive nature.

Additional groups of rats (12/sex/group) were treated in parallel with those of the previous study in order to assess neurotoxicity of cyclohexane in FOB, motor activity and neuropathology tests (Haskell Laboratory 1996c). Neurobehavioral evaluations were conducted prior to exposure and at week 4, 8, and 13. During each evaluation period FOB was performed prior to the motor activity test. At the end of the study, 6 rats/sex/group were selected for neuropathology, the controls and 7,000 ppm tissues selected were examined, and the intermediate dose tissues were saved. Neurological lesions were also assessed by examining sections of the brain, spinal cord, sciatic nerve, gasserian ganglia, cervical and dorsal root fibers and ganglia, cervical and lumbar ventral root fibers and gastrocnemius muscle.

Similar to the main 90 day study described previously, a sedative effect was observed at doses of 2,000 ppm and higher characterized by a decrease in the mean response to an alerting stimulus. This effect was transient since no effects were observed immediately after removal from the exposure chamber. No effects were observed during the FOB and motor activity assessment. Histologically, no treatment-related findings were observed, the only lesions observed being identical in character and severity to those observed in controls. These have already been described as occurring spontaneously in the rat. The NOAEL for neurotoxicity was 500 ppm based on the transient sedative effect observed at 2,000 ppm and higher.

A 13 week inhalation toxicity study (Haskell Laboratory, 1996b; Malley *et al*., 2000) in mice was also performed following a 2-week range finding study in mice. The study

was comparable in experimental conditions to that performed on rats. For neurologic effects, a NOAEL of 500 ppm was determined based on signs of sedation observed in animals at 2,000 ppm. The NOAEL for systemic effects was 2,000 ppm based on effects observed in the liver at 7,000 ppm (increase in absolute and relative liver weights).

n-Hexane

A fair number of subchronic inhalation studies with n-hexane have been performed, particularly concerning the neurotoxic potential of n-hexane in rats (Ono *et al.*, 1982; Pryor *et al.*, 1983; Howd *et al.*, 1983; Cavender *et al.*, 1984a,b; Huang, 1989, 1992; Biodynamics, 1978, IRDC, 1992; NTP, 1991). Three of these key studies are described.

The subchronic toxicity of n-hexane is of some concern due to reports of treatment related peripheral neuropathy. A key 13-week inhalation study of n-hexane in male and female rats provided strong evidence for treatment related peripheral neuropathy. (Cavender *et al*. 1984a,b). Male and female F344 rats (15/sex/group) were exposed to n-hexane at 0, 3,000, 6,500, and 10,000 ppm for 6 hours/day, 5 days/week for 13 weeks. There were no n-hexane-related clinical signs of toxicity, effects on food consumption, ophthalmological findings, or changes in neurological function. However, there was a lowering of the urinary pH in high-dose males. There were increased organ/body weight ratios for liver, kidney, and testis in high-dose males and kidney in mid-dose males. Histopathological examination of the tibial nerves revealed paranodal axonal swelling in mid- and high-dose males. n-Hexane is metabolized to 5-hydroxy-2-hexanone and 2,5-hexanedione *in vivo* (DiVincenzo *et al*., 1976). These two metabolites are believed to be responsible for the neurotoxicities associated with n-hexane exposure. There is evidence to show that 2,5-hexanedione is more persistent in peripheral nerve tissue than the parent n-hexane (Bus *et al*., 1981).

In a 16-week inhalation toxicity study, male Wistar rats (8/group) were exposed to 0, 500, 1,200, or 3,000 ppm n-hexane for 12 hours/day, 7 days/week (Huang *et al.*, 1989). Motor nerve conduction velocity was measured in the tail nerve along with body weight before exposure and after 4, 8, 12, and 16 weeks of exposure. One animal from each group was sacrificed after 16 weeks of exposure for histopathological evaluation of the nerve fibers in the tail. In addition, nerve-specific proteins (i.e. enolase and β-S100), involved in processes such as cell-cell communication, cell growth, intracellular signal transduction, and development and maintenance of the central nervous system, were measured. Statistically significant reductions in body weight gain were observed in a for the mid-dose (at 12 weeks) and high-dose (at 8 weeks) rats. Neurological deficits (i.e. reduction in grip strength and comparative slowness of motion) in mid- and high-dose rats were noted from 12 weeks of exposure. No hind-limb paralysis was observed by the time of sacrifice. A reduction in motor nerve conduction velocity, statistically significant during weeks 8-16, was seen with mid and high-dose rats. In addition increased incidence of paranodal swelling, along with some evidence of demyelination and remyelination was present in the peripheral nerves with 1,200 and 3,000 ppm. These histopathologic findings were most sever in the high-dose group. Dose-dependent biochemical changes included reductions in nervous system-specific proteins, particularly the β-S100 protein in tail nerve fibers which was reduced by approximately 75% at all dose levels. Under the conditions of this study, the NOAEL

was 500 ppm based on the neurophysiologic deficits and histopathologic effects seen with 1,200 and 3,000 ppm.

A 13-week inhalation study of n-hexane in mice was conducted in 1991 by the NTP. Groups of 10 mice/sex/group were exposed to 0, 500, 1,000, 4,000, or 10,000 ppm n-hexane 6 hours/day, 5 days per week for the duration of the study. A second group of 10 mice was exposed at 1,000 ppm for 22 hours/day, 5 days/week for the duration of the study. Separate groups of 8 mice/sex/group received identical treatments but were subjected to neurobehavioral tests before the start of dosing then again after 6 and 13 weeks of exposure. Four males and four females were randomly selected from the 0, 1,000 ppm extended duration, and 10,000 ppm exposure groups for histopathological examination of the spinal cord and tibial nerves. Animals were observed daily for signs of clinical toxicity and weighed weekly.

A full necropsy was performed at sacrifice, weights of the major organs were recorded, and histopathological evaluations were carried out at term on a variety of excised organs and tissues. The liver was examined only in the males of all exposure groups. Animals exposed to 10,000 ppm n-hexane exhibited some signs of nasal irritation and all animals survived to term. Relative liver, kidney, and heart weights appeared to be increased compared with controls in exposed females. In addition, females exposed to 10,000 ppm 6 hours/day and 1,000 ppm for 22 hours/day exhibited neurobehavioral deficits with a reduction in locomotor activity. There was an increased incidence of paranodal axonal swelling in high-dose or extended exposure duration mice. It was concluded that n-hexane caused minimal toxicity to the nervous system and/or respiratory system at 1,000 ppm and above indicating a NOAEL of 500 ppm.

2,4-dimethyl pentane (n-Heptane; 2-methylpentane; 3-methylpentane; 2,2,4-trimethylpentane)

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 2,970 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 2,970 ppm and the NOAEL for systemic toxicity was 2,970 ppm.

In an inhalation neurotoxicity study, Sprague-Dawley rats (6-9 males/dose group) were exposed by inhalation to air or 1,500 ppm n-heptane, 2-methylpentane or 3-methylpentane for 9 hours/day, 5 days/week for 30 or 14 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. However, a significant decrease in weight gain of rats treated with 1500 ppm 2-methylpentane was noted. Differences between mean values for hind limb spreads observed in treated animals and controls were not statistically significant. No histological signs of giant axonal degeneration were noted in rats treated at 1,500 ppm. Under the condition of this test, the NOAEL for repeated dose toxicity was considered to be 1,500 ppm.

Short et al. (1989) exposed male and female F344 rats for 6 hours/day, 5 days/week to 0 (control) or 50 ppm (234 mg/m3) 2,2,4-trimethylpentane via inhalation to characterize the pathogenesis of α2u-globulin nephropathy. Body weight was the only other endpoint evaluated. Male rats (three/group) were exposed for 3, 10, 22, or 48 weeks and female rats (three/group) were exposed for 3, 11, 23, or 50 weeks. The presence of α2u-globulin in renal tissues was measured by immunohistochemistry at each time point. Cell proliferation was determined by measuring incorporation of thymidine administered during the last exposure week of each exposure period. No significant differences were noted in body weights or in absolute or relative kidney weights in male or female rats at any time point with or without treatment. A significant increase in detectable α2u-globulin in exposed males, but not females, was observed after 3 weeks of exposure, compared with controls ($p < 0.05$). This effect persisted throughout each of the exposure periods and did not vary with time. In addition, the subcellular localization of α2u-globulin corresponded with hyaline droplets in serial sections. Examination of the P2 segment of the proximal tubule of the kidney showed an increase of up to 11-fold in cell turnover in male rats, starting at 3 weeks and persisting throughout the study. This proliferative response closely paralleled the extent and severity of immunohistochemically detectable α2u-globulin in the P2 segment. Neither cytotoxicity nor α2u-globulin were noted in the P1 or P3 segments. Increased numbers of proximal tubules affected by chronic progressive nephrosis (CPN) were noted in male rats exposed to 2,2,4-trimethylpentane for 22 (7.7 CPN foci/section) or 44 weeks (12.3 CPN foci/section), compared with controls (0.4 and 5.0 CPN foci/section, respectively). These lesions contained highly proliferative epithelial cells. Control and exposed female rats exhibited no evidence of α2u-globulin-related nephropathy, increases in cell turnover, or chronic nephrosis. In both control and exposed females, the number of CPN foci per kidney section was 0.3 at 23 weeks and 1.3 at 50 weeks.

In summary, data are available to adequately characterize the repeated dose toxicity of C4-6 IRRP Fraction stream. The C4-6 IRRP Fraction stream is expected to have a low order of repeated dose toxicity although some concern is noted based on evidence for peripheral neuropathy as a result of n-hexane exposure. No further testing is proposed.

Reproductive and Developmental Toxicity

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

Cyclohexane

A two-generation reproductive toxicity study of inhaled cyclohexane vapor was conducted with CD BR rats (Haskell Laboratory, 1997c) according to OECD and US

Environmental Protection Agency guidelines. In this study, weanling F0 rats (30/sex/group) inhaled cyclohexane vapor at 0, 500, 2,000, or 7,000 ppm 5 day/week and 6 h/day. Exposure duration was 10 weeks before mating until sacrifice of the P1 generation and 11 weeks before mating until sacrifice of the F1 generation. Gravid females were not exposed from day 21 of gestation until day 4 of lactation. From day 5 of lactation until weaning the neonates were potentially exposed by maternal milk; no other exposure was administered. At post partum day 25, thirty F1 animals/sex/dose were chosen to produce the next generation, treatment was continued 11 weeks before mating and during gestation. Fertility parameters were calculated. From 500 ppm, there was an increased incidence of diminished response to a stimulus during exposure, this finding being significant at 2,000 ppm and higher. At 7,000 ppm, major effects were observed on body weight, body weight gain and food efficiency. A decrease in mean body weight was seen with F1 male rats, P1 and F1 females during pre-mating, P1 females throughout gestation, and F1 females during lactation. A decrease in mean body weight gain was observed with F1 male rats and P1 and F1 females during pre-mating; however no reductions were seen for P1 females during gestation, suggesting that the reduction in mean gestation body weight was probably due to pre-existing body weight deficits established during the pre-mating period. The same findings were also seen with the F1 generation. A decrease in mean food efficiency of P1 and F1 females during lactation and a decrease in food consumption of P1 females during lactation were also observed. Effects on reproduction were limited to a decrease in mean pup weight for both the F1 and F2 generations at the high dose which was significant between post partum day 7 and 25 during which time pups were fed only maternal milk indicating the effect is due to cyclohexane via lactation. There was a slight increase in the incidence of pro-static inflammation at 7,000 ppm in P1 and F1 adults, but this was considered incidental due to the lack of severity and the reported common occurrence in rats. There was a slight but significant decrease in the mean percentage of animals born alive in the F1 litters dosed with 7,000 ppm, but given that the value was still in the range of historical controls and that this effect was not dose-related, this was not considered biologically significant.

The NOAEL for adult reproductive toxicity was 2,000 ppm based on decreases in pup body weight observed at 7,000 ppm. The systemic NOAEL for this study was 500 ppm based on sedative effects observed at 2,000 ppm and higher.

In another EPA and OECD guideline study, the developmental toxicity of cyclohexane in rats was tested (Haskell Laboratory, 1997a). As a pilot study, four groups of eight pregnant CDBR rats were exposed whole-body to concentrations of 0, 3,000, 6,000, or 9,000 ppm cyclohexane from gestational day 7 to 16. Dams were sacrificed on day 22 and examined for gross pathologies; implantations and resorptions were counted and their relative positions recorded; fetuses were weighed and examined externally for alterations. Maternal effects were limited to a reduction in overall maternal bodyweight gain, overall food consumption and diminished response of animals to a sound stimulus during exposure to 6,000 ppm and higher. No effects were observed in the pups. The NOAEL was 3,000 ppm for the dams and 9,000 ppm for the pups.

This pilot study served as a range-finding study used to design a more complete study which was carried out during the 90 day inhalation study previously described (Haskell Laboratory, 1997b). Four groups of CD BR rats were exposed whole body to cyclohexane at concentrations of 0, 500, 2,000 or 7,000 ppm from gestational day 7 to

16. Animals were sacrificed on day 22 and examined. Findings were limited to the dams and included a diminished response of the animals to a sound stimulus while in the chamber during exposure and at 2,000 ppm or higher and reductions in overall body weight gain and food consumption throughout the treatment period. A slight but significant decrease in implantation number with the number of corpora lutea unchanged was seen when compared with controls. This finding was consistent with slight pre-implantation losses and can be considered as not treatment-related since there was no treatment during the pre-implantation period. The NOAEL was 500 ppm for maternal toxicity and 7,000 ppm for developmental toxicity considering the lack of toxic effects noted.

n-Hexane

The developmental toxicity of n-hexane was assessed using timed-pregnant (30 animals per group) and virgin (10 animals per group) Sprague-Dawley rats exposed to 0 (filtered air), 200, 1,000, and 5,000 ppm n-hexane vapor in inhalation chambers for 20 hours per day for a period of 14 consecutive days (Mast, 1987). Spermpositive females were exposed on gestation days (GD) 6-19 and virgins were exposed concurrently for 14 consecutive days. Maternal toxicity, manifested as a reduction in extra-gestational maternal weight gain, was observed at all exposure levels, and was statistically significant for the 5,000 ppm exposure group. Extra-gestational maternal weight gain (calculated from GD 0 to GD 20) relative to control animals was reduced for the 200, 1,000, and 5,000 ppm exposure groups. Cumulative weight gain (CWG) for dams in the 1,000 and 5,000 ppm exposure groups was significantly reduced with respect to controls by GD 20. The CWG for the 5,000 ppm was also significantly reduced with respect to controls by GD 13.

Comparison of n-hexane exposed groups with the control group (0 ppm) indicated that gestational exposure to n-hexane did not result in an increase in the incidence of intrauterine deaths or in the incidence of fetal malformations. A statistically significant reduction in fetal body weight relative to controls was observed for males at the 1,000 and 5,000 ppm exposure levels. Female weights were also reduced with respect to controls for these exposure levels, but the reduction was statistically significant for only the 5,000 ppm group. Gravid uterine weight was also significantly less than controls for the 5,000 ppm exposure groups. A statistically significant increase in the mean percent incidence per litter of reduced ossification of sternebrae 1-4 was observed for the 5,000 ppm group, and was positively correlated with exposure concentration. This increased incidence of reduced ossification in the sternebrae, and the reduction in fetal body weight at the 5,000 ppm level, may have been inter-related manifestations of slight growth retardation.

No major abnormalities were found in any of the fetuses. Variations observed included dilated ureter, renal pelvic cavitation, supernumerary ribs, and reduced skeletal ossifications at several sites. The increase in mean percent incidence per litter of reduced ossification of sternebrae 1-4 was statistically significant for the highest exposure concentration, and the increase was positively correlated with increasing exposure concentration. The NOAEL for developmental toxicity was 200 ppm.

The effect of n-hexane on the male reproductive system when administered via the inhalation route was examined by exposing male Sprague-Dawley rats (12-39/group) to

5,000 ppm n-hexane in either a single 24 hour exposure, repeated 16 hour/day exposures for up to 8 days, or repeated 16 hour/day exposures, 6 hours/day for up to 6 weeks (De Martino *et al.*, 1987). Rats exposed to 5,000 ppm n-hexane displayed some evidence of neuropathy such as paralysis. Focal degeneration of spermatocytes and exfoliation of elongated spermatids was also observed in response to treatment. Early meiotic prophase spermatocytes and transitional spermatocytes as well as those undergoing meiotic metaphase appeared to be more susceptible to the action of n-hexane than pachytene spermatocytes. Rats exposed repeatedly to 5,000 ppm n-hexane over a 6-week period showed complete atrophy of the seminiferous tubules. In addition, a reduction in food consumption and body weight gain accompanied by signs of incipient neuropathy were seen with repeated n-hexane exposure. A wide range of testicular lesion did not complete resolve during a recovery period even though body weights and clinical symptoms improved.

Timed-pregnant (~33 females per group) and virgin (10 females per group) Swiss (CD-1) mice were exposed to 0, 200, 1,000, and 5,000 ppm n-hexane (99.2% purity) vapor in inhalation chambers, 20 h/day, for a period of 12 consecutive days (Mast *et al.*, 1988b). Plug-positive females were exposed on GD 6-17. Maternal body weight at sacrifice (GD 18) and total cumulative weight gain for dams in the 5,000 ppm exposure group were significantly reduced with respect to controls; however, this was due to an exposure correlated reduction in gravid uterine weight, not to a decrease in extragestational gain. An exposure-correlated decrease in the gravid uterine weight to extragestational weight gain ratio (significant for the 5,000 ppm group) occurred in the absence of an effect on placental weight.

Gestational exposure to n-hexane resulted in an increase in the number of resorbed fetuses for all exposure groups relative to the control group; however, the increases were not directly correlated to exposure concentration. The differences were statistically significant for the 200-ppm group with respect to total intrauterine death (early plus late resorptions), and with respect to late resorptions for the 5,000 ppm group. A small, but statistically significant, reduction in female (but not male) fetal body weight relative to the control group was observed at the 5,000 ppm exposure level. There were no exposure-related increases in any individual fetal malformation or variation, nor was there any increase in the incidence of combined malformations or variations.

Gestational exposure of CD-1 mice to n-hexane vapors appeared to cause a degree of concentration-related developmental toxicity in the absence of overt maternal toxicity, but the test material was not found to be teratogenic. This developmental toxicity was manifested as an increase in the number of resorptions per litter for all exposure levels, and as a decrease in the uterine: extra-gestational weight gain ratio at the 5,000 ppm exposure level. Because of the significant increase in the number of resorptions at the 200-ppm exposure level, a NOEL for developmental toxicity was not established for exposure of mice to 200, 1,000, or 5,000 ppm n-hexane vapors.

The available data on predominant constituents of the C4-6 IRRP Fraction 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 C4-6 IRRP Fraction stream is expected to have a low order of reproductive and developmental toxicity.

Conclusion

Mammalian toxicology data on three constituents of the C4-6 IRRP Fraction stream, cyclohexane, n-hexane and n-heptane 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 with the exception of n-hexane induced peripheral neuropathy for which some concern is noted. Reproductive and developmental toxicity potential is considered low. The weight of the evidence for *in vitro* and in vivo genotoxicity testing of cyclohexane, n-hexane, and n-heptane indicates no strong evidence for genotoxicity. 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 cyclohexane.

TOXICITY ENDPOINT		RESULTS	REFERENCE
Acute	Inhalation	LC50 > 32.88 mg/L	Phillips Petroleum Company, 1982b
	Oral	LD50 > 5,000 mg/kg	Phillips Petroleum Company, 1982a
	Dermal	LD50 > 2,000 mg/kg	Phillips Petroleum Company, 1982c
Irritation	Skin	Skin irritant	Phillips Petroleum Company, 1982d; Jacobs and Martens, 1987
	Eye	Minimal irritant	Phillips Petroleum Company, 1982e,f
Sensitization		Not a dermal sensitizer	White Eagle Toxicology Labs, 1996; EU Risk Assessment, 2004
Repeated Dose		Rat: NOAEL = 2,000 ppm	Haskell Laboratories, 1996a
Reproductive		NOAEL for Adult Reproductive toxicity = 2,000 ppm NOAEL for Systemic toxicity = 500 ppm	Haskell Laboratories, 1997e
Developmental		NOAEL for maternal toxicity = 500 ppm (rat, mouse) NOAEL developmental toxicity = 7,000 ppm (rat)	Haskell Laboratories, 1997c,d
Neurotoxicity		For FOB and motor activity assessment, very few effects were observed. Cyclohexane did not induce neurobehavioral effects.	TNO, 1998b
Genotoxicity	*In vitro* Ames *Salmonella* assay	Negative	Mortlemans *et al*., 1986
	In vitro chromosome aberration		
	In vivo micronucleus	Negative - Cyclohexane was not clastogenic to rat bone marrow	American Petroleum Institute, 1982

Table 9. Mammalian toxicity endpoint summary for n-hexane.

TOXICITY ENDPOINT		RESULTS	REFERENCE
Acute	Inhalation	LC50 = 48,000 ppm	HEDSET, 1982
	Oral	LD50 = 28,720 mg/kg	HSDB, 2005
	Dermal		
Irritation	Skin	Mild Irritant – Dermal exposure can lead to peripheral neuropathy in humans	Nomiyama *et al*., 1973; Spencer *et al*., 1980 & 1987
	Eye		
Sensitization			
Repeated Dose		Rat: NOAEL = 500 ppm Mouse: NOAEL = 500 ppm	Huang *et al*., 1989; NTP, 1991
Reproductive			
Developmental		NOAEL for maternal toxicity = 200, 1,000 ppm (rat, mouse) NOAEL developmental toxicity = 200 ppm (rat), LOAEL developmental toxicity = 200 ppm (mouse)	Mast *et al*., 1987; 1988
Neurotoxicity		Evidence for peripheral neuropathy	Cavender *et al*., 1984a,b
Genotoxicity	*In vitro* Ames *Salmonella* assay	Negative	NTP, 1991
	In vitro chromosome aberration	Negative	Daughtrey *et al*., 1994; NTP, 1991
	In vivo micronucleus	Negative – n-hexane was not clastogenic to mouse bone marrow Positive – n-hexane was clastogenic to albino rat bone marrow	Shelby and Witt, 1995 Hazleton Laboratories, 1992

Table 10. Mammalian toxicity endpoint summary for 2,4-Dimethylpentane (n-heptane, iso-octane, 2-methylpentane, 3-methylpentane, 2,2,4-trimethylpentane).

TOXICITY ENDPOINT		RESULTS	REFERENCE
Acute	Inhalation	LC50 > 29.29 mg/L LC50 < 32,000ppm (iso-octane)	HEDSET, 1982 Swann *et al.,* 1974
	Oral		
	Dermal		
Irritation	Skin		
	Eye		
Sensitization			
Repeated Dose		Rat: NOAEL = 2,970 ppm (n-heptane) Rat: NOAEL = 1500 ppm (n-heptane, 2-methylpentane, 3-methylpentane) Rat: LOAEL = 50 ppm (234 mg/m3) (2,2,4-trimethylpentane	American Petroleum Institute, 1980 Frontali *et al.,* 1981 Short *et al.,* 1989
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 - mitotic gene conversion assay using *Saccharomyces cerevisiae (n-heptane)* Negative – Sister chromatid exchange test (2,2,4-trimethylpentane)	Brooks *et al*., 1982 Richardson *et al*., 1986
	In vivo micronucleus	Negative - Unscheduled DNA Synthesis was not induced with 2,2,4-trimethylpentane Negative – No increase in double strand breaks and no induction of poly-ADP-ribosylation with 2,2,4-trimethylpentane	Loury *et al*., 1986 *McLaren et al.,* 1994

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 C4-6 IRRP Fraction stream. The three constituents were n-hexane, 2,4-dimethylpentane, and cyclohexane. Adequate data for n-hexane, 2,4-dimethylpentane, and cyclohexane are shown in Table 11.

Table 11. n-hexane, 2,4-dimethylpentane, and cyclohexane 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}
Hexane	**A**	**A**	**A**	**A**	**A**	**A**	**C**	**A/C**	**C**	**T**	**T**	**C**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
2,4-dimethylpentane	**A**	**A**	**A**	**A**	**-**	**-**	**C**	**A/C**	**C**	**T**	**T**	**C**	**A/C**	**A**	**A**	**A**	**A**	**A**	**A**
Cyclo-hexane	**A**	**A**	**-**	**A**	**A**	**A**	**A/C**	**A/C**	**A/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

V. REFERENCES

Abernethy, S., Bobra, A.M., Shiu, W.Y., Wells, P.G. and Mackay, D. (1986). Acute lethal toxicity of hydrocarbons and chlorinated hydrocarbons to two plank tonic crustaceans: the key role of organism-water partitioning, Aquatic Toxicology 8, 163-174.

American Petroleum Institute (1980). A 26-week inhalation toxicity study of heptane in the rat. Unpublished Report.

American Petroleum Institute (1982). Mutagenicity evaluation of certified cyclohexane in the rat bone marrow cytogenetics assay. API Medical Research Publication Number 29-32357, Final Report. Litton Bionetics Inc.

American Petroleum Institute (1986). Mutagenicity evaluation of certified cyclohexane. API Final Report. Project No. PS-4-LBI (503-0), Litton Bionetics Inc.

American Public Health Association (1971). Standard methods for the examination of water and waste water. 13th ed. American Public Health Association, Inc., Washington, DC, USA.

Atkinson, R. (1989). J. Phys. Chem. Ref. Data Monograph 1.

Biodynamics Inc. (1978). 26-Week inhalation toxicity study of n-hexane in the rat. EPA Document No. FYI-AX-1081-0137; NTIS No. OTS0000137-0.

Boublik, T., Fried, V. and Hala, E. (1984). The vapour pressures of pure substances. 2nd Revised edition, Elsevier, Amsterdam.

Brooks T, Meyer, A. and Hutson, D. (1982). The genetic toxicology of some hydrocarbon and oxygenated solvents. Mutagenesis. 3, 227-232.

Bus, J.S., White, E.L., Gilliers, P.J. *et al*. (1981). Tissue distribution of n-hexane, methyl-n-butyl ketone and 2,5-hexanedione in rats after single or repeated inhalation exposure to hexane. Drug Metab Dispos. 9, 386-387.

Cavender, F.L., Casey, H.W., Salem, H. *et al*. (1984a). A 13-week vapor inhalation study of n-hexane in rats with emphasis on neurotoxic effects. Fundam Appl Toxicol 4 (Part 1), 191-201.

Cavender, F.L., Casey, H.W., Gralla, E.J. *et al*. (1984b). The subchronic inhalation toxicity of n-hexane and methyl ethyl ketone. Advances in Modern Environ Toxicol. 6, 215-231.

Chemicals Inspection and Testing Institute (CITI) (1992). Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan. CITI (ed.). Chemical Products Safety Division, Basic Industries Bureau, Ministry of International Trade and Industry, Japan. Japan Chemical Industry Ecology-Toxicology and Information Center.

Chao, J., Lin, C.T. and Chung, T.H. (1983). Vapor pressure of coal chemicals. J. Phys. Chem. Ref. Data. 12, 1033-1063.

Daubert, T. and Danner, R. (1989). Physical and thermodynamic properties of pure chemicals: Data compilation. Design Institute for Physical Property Data, American Institute of Chemical Engineers. Hemisphere Publishing Corp., New York, NY, USA.

Daughtrey, W.C., Putman, D.L., Duffy, J. *et al*. (1994b). Cytogenetic studies on commercial hexane solvent. J Appl Toxicol. 14, 161-165.

DeMartino, C., Malorni, W., Amantini, M.C. *et al*. (1987). Effects of respiratory treatment with n-hexane on rat testis morphology. I. A light microscopic study. Exp Mol Pathol. 46, 199-216.

Deichmann, W.B. and Le Blanc, T.J. (1943). Determination of the approximate lethal dose with about six animals. J Ind Health Toxicol. 25, 415-417.

Di Toro, D.M., McGrath, J.A., and Hansen, D.J. (2000). Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and Tissue. Envir Toxicol & Chem, 19,1951-1970.

DiVincenzo, G.D., Kaplan, C.J. and Dedinas, J. (1976). Characterization of the metabolites of methyl n-butyl ketone, methyl iso-butyl ketone, and methyl ethyl ketone in guinea pig serum and their clearance. Toxicol Appl Pharmacol. 36, 511-522.

ECOSAR v0.99h (2004) in EPI Suite™, U.S. EPA (2000). Estimation Program Interface Suite, v3.12. Syracuse Research Corporation, Syracuse, NY, USA.

EMBSI (1995). Ready Biodegradability: OECD 301F Manometric Respirometry Test. ExxonMobil Biomedical Sciences Inc. Project No. 103394A. Final Report.

EMBSI (1996). Ready Biodegradability: OECD 301F Manometric Respirometry Test. ExxonMobil Biomedical Sciences Inc. Project No. 116894A. Final Report.

EMBSI (1998). Algal inhibition test. ExxonMobil Biomedical Sciences, Inc., Project No. 114267, Final Report, April 1998.

EMBSI (2001). Fish acute toxicity test. ExxonMobil Biomedical Sciences, Inc., Project No. 161858, Final Report, February 2001.

Frontali, N., Amantini, M., Spagnolo, A., Guarcini, A., Saltari, M., Brugnone, F. and Perbellini, L. (1981). Experimental neurotoxicity and urinary metabolites of the C5-C7 aliphatic hydrocarbons used as glue solvents in shoe manufacture. Clin Toxicol. 18,1357-1367.

Geiger, D.L., Brooke, L.T., Call, D.J. (1987). Acute toxicities of organic chemicals to fathead minnows (Pimephales promelas), Vol. 5, Centre for Lake Superior Studies, University of Wisconsin Superior, WI, 332 p.

Haines, J. and Alexander, M. (1974). Microbial degradation of high-molecular-weight alkanes. Appl Microbiol. 28,1084-1085.

Hansch, C., Leo, A. and Hoekman, D. (1995). Exploring QSAR - Hydrophobic, electronic, and steric constants. American Chemical Society. Washington, DC, USA.

Haskell Laboratory (1995). Two week inhalation range-finding study with cyclohexane in rats. Laboratory Report No. 40-95.

Haskell Laboratory (1996a). 90-Day inhalation toxicity study with cyclohexane in rats. Laboratory Report No. 298-96.

Haskell Laboratory (1996b). 90-Day inhalation toxicity study with cyclohexane in mice. Laboratory Report No. 17-96.

Haskell Laboratory (1996c). 90-Day inhalation neurotoxicity study with cyclohexane in rats. Laboratory Report No. 752-95.

Haskell Laboratory (1997a). Pilot inhalation developmental toxicity of cyclohexane in rats. Laboratory Report No. 18-96.

Haskell Laboratory (1997b). Inhalation developmental toxicity study of cyclohexane in rats. Laboratory Report No. 881-96.

Haskell Laboratory (1997c). Reproductive and fertility effects with cyclohexane. Inhalation multigeneration reproductive study in rats. Laboratory Report No. 409-96.

Harris J (1982a). Rate of aqueous photolysis. In: Handbook of Chemical Property Estimation Methods. Chapter 8. Edited by WJ Lyman, WF Reehl and DH Rosenblatt. McGraw-Hill Book Company, New York, NY, USA.

Harris, J. (1982b). Rate of hydrolysis. In: Handbook of Chemical Property Estimation Methods. Chapter 7. Edited by WJ Lyman, WF Reehl and DH Rosenblatt. McGraw-Hill Book Company, New York, NY, USA.

Hazelton Laboratories (1992). Initial submission: in vivo and in vitro mutagenicity studies n-hexane (hexane UV) (final report) with attachments and cover letter dated 020592. Submitted under Section 8ECP of TSCA. EPA document No. 88-920000955; NTIS No. OTS0535721.

HEDSET (1982). Acute inhalation toxicity test, n-heptane, Final Report.

Howd, R., Rebert, C., Dickinson, J. *et al*. (1983). Comparison of the rates of development of functional hexane neuropathy in weanling and young adult rats. Neurobehav Toxicol Teratol. 5, 63-68.

HSDB (Hazardous Substances Data Bank) (2005). n-Hexane. National Library of Medicine, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD. Available from: <jttp://toxnet.nlm.nih.gov>

Huang, J., Kato, K., Shibata, E. *et al*. (1989). Effects of chronic n-hexane exposure on nervous system-specific and muscle-specific proteins. Arch Toxciol. 63, 381-385.

Huang, J., Shibata, E., Kato, K., *et al*. (1992). Chronic exposure to n-hexane induces changes in nerve-specific marker proteins in the distal peripheral nerve of the rat. Human & Exper Toxicol. 11, 323-327.

IRDC (International Research and Development Corporation). (1992a). 6-Month continuous inhalation exposures of rats to hexane mixtures – Phase I. EPA Document No. FYI-0892-0901; NTIS No. OTS0000901K6.

IRDC (1992b). 6-Month continuous inhalation exposures of rats to hexane mixtures – Phase II. EPA Document No. FYI-0892-0901; NTIS No. OTS0000901K5.

Ishidate, M., Sofuni, T., Yoshikawa, K., *et al*. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol. 22, 623-636.

Jacobs, G. and Martens, M. (1987). Evaluation of the test method for skin irritation as prescribed by OECD and EC. J Toxicol-Cut Ocular Toxicol. 6, 215-225.

Kimura, E.T., Ebert, D.M., and Dodge, P.W. (1971). Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol. 19, 699-704.

Klimisch, H., Andreae, M. and Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Reg Toxicol & Pharm. 25,1-5.

Kubinski, H., Gutzke, G.E., and Kubinski, Z.O. (1981). DNA cell-binding assay for suspected carcinogens and mutagens. Mut Res. 89, 95-136.

Lide, D. (2003). CRC Handbook of Chemistry and Physics. 84th Edition. CRC Press, New York, NY, USA.

Litton Bionetics, Inc. (1979). Mutagenicity evaluation of n-hexane in the mouse dominant lethal assay [final report]. EPA Document No. FYI-AX-1081-0137. NTIS No. OTS0000137-0.

Loury, DJ; Smith-Oliver, T; Strom, S; et al. (1986) Assessment of unscheduled and replicative DNA synthesis in hepatocytes treated in vivo and in vitro with unleaded gasoline or 2,2,4-trimethylpentane. Toxicol Appl Pharmacol 85:11–23.

Mackay, D. (1998a). Level I fugacity-based environmental equilibrium partitioning model, Version 2.1 (16-bit). Environmental Modelling Centre, Trent University, Ontario, Canada.

Mackay, D. (1998b). Level III fugacity-based environmental equilibrium partitioning model, Version 2.1 (16-bit). Environmental Modelling Centre, Trent University, Ontario, Canada.

Malley, L.A., Bamberger, J.R., Stadler, J.C., Elliott, G.S., Hansen, J.F., Chiu, T., Grabowski, J.S. and Pavkov, K.L. (2000). Subchronic toxicity of cyclohexane in rats and mice by inhalation exposure. Drug Chem Tox. 23, 513-537.

Mast, T. (1987). Inhalation developmental toxicology studies: Teratology study of n-hexane in rats [final report]. Public Health Service, US Department of Health and Human Services; NTP TER90082. Prepared by the Pacific Northwest Laboratory, Richland, WA, for the National Toxicology Program, National Institute for Environmental Health Services, Research Triangle Park, NC; PNL-6453.

Mast, T. (1988a). Inhalation developmental toxicology studies: Teratology study of n-hexane in mice [final report]. Public Health Service, US Department of Health and Human Services; NTP TER90083. Prepared by the Pacific Northwest Laboratory, Richland, WA, for the National Toxicology Program, National Institute for Environmental Health Services, Research Triangle Park, NC; PNL-6590.

Mast, T., Rommerein, R., Evanoff, J. *et al.* (1988b). Inhalation reproductive toxicology studies: male dominant lethal study of n-hexane in Swiss (CD-1) mice. Prepared by the Pacific Northwest Laboratory, Richland, WA, for the National Toxicology Program, National Institute for Environmental Health Services, Research Triangle Park, NC; PNL-6679.

McAuliffe, C. (1966). Solubility in water of paraffin, cycloparaffin, olefin, acetylene, cycloolefin and aromatic hydrocarbons. J. Phys. Chem. 76. 1267-1275.

McCarty, L.S. and Mackay, D. (1993). Enhancing ecotoxicological modeling and assessment. Environ Sci Technol. 27,1719-1728.

McCarty, L.S., Mackay, D., Smith, A.D., Ozburn, G.W. and Dixon, D.G.. (1991). Interpreting aquatic toxicity QSARs: The significance of toxicant body residues at the pharmacologic endpoint. In: WSAR in Environmental Toxicology - IV. J.L.M. Hermens and A. Opperhuizen, eds. Elsevier.

McLaren, J; Boulikas, T; Vamvakas, S. (1994) Induction of poly(ADP-ribosyl)ation in the kidney after in vivo application of renal carcinogens. Toxicology 88:101–112.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ Mutagen. 8, 1-119.

National Toxicology Program (NTP). (1991). Toxicity studies of n-hexane in B6C3F1 mice (inhalation studies). Public Health Service, US Department of Health and Human Services; TOX-2.

Neely, W. (1985). Hydrolysis. In: W. B. Neely and G. E. Blau, eds. Environmental Exposure from Chemicals. Vol I., pp. 157-173. CRC Press, Boca Raton, FL, USA.

Nomiyama, K., Yoshida, T. and Yanagisawa, H. (1973). Percutaneous absorption of n-hexane caused severe polyneuropathy. Proc 46th Ann Jpn Assoc Ind Health. p.420.

Ono, Y., Takeuchi, Y. Hisanaga, N. *et al*. (1982). Neurotoxicity of petroleum benzine compared with n-hexane. Int Arch Occup Environ Health 50, 219-229.

Phillips Petroleum Company (1982a). Acute oral toxicity study in rats. Cyclohexane. Final Report, Project No. 652-127.

Phillips Petroleum Company (1982b). Acute inhalation toxicity test cyclohexane. Final Report, Project No. 652-1148.

Phillips Petroleum Company (1982c). Acute dermal toxicity study in rabbits. Cyclohexane. Final Report, Project No. 652-122.

Phillips Petroleum Company (1982d). Primary skin irritation study in rabbits. Cyclohexane. Final Report, Project No. 652-118.

Phillips Petroleum Company (1982e). Washed primary eye irritation study in rabbits. Cyclohexane. Final Report, Project No. 652-117.

Phillips Petroleum Company (1982f). Unwashed primary eye irritation study in rabbits. Cyclohexane. Final Report, Project No. 652-116.

Phillips Petroleum Company (1982g). Mouse lymphoma forward mutation assay. Cyclohexane. Final Report, Project No. 652-120.

Phillips Petroleum Company (1982h). In vitro sister chromatid exchange in chinese hamster ovary cells. Cyclohexane. Final Report, Project No. 652-121.

Perocco, P., Bolognesi, S. and Aberghini, W. (1983). Toxic activity of seventeen industrial solvents and halogenated compounds of human lymphocytes cultured in vitro. Toxicol Lett. 16, 69-75.

Prince, R.C., T.F. Parkerton, C. Lee. (2007). The primary aerobic biodegradation of gasoline hydrocarbons. Environmental Science and Technology. 41: 3316-3321.

Prince, R.C., C. Haitmanek, C. Lee. (2008). The primary aerobic biodegradation of biodiesel B20. Chemosphere, 71(8): 1446-1451.

Pryor, G., Dickinson, J., Howd, R. *et al*. (1983). Neurobehavioral effects of subchronic exposure of weanling rats. Neurobehav Toxicol Teratol. 5, 47-52.

Ramos, E.U., Vaes, W.H.J., Verhaar, H.J.M. and Hermens, J.L.M.. (1998). Mechanism of narcosis: A proposed mechanism for narcosis mode of action. In: Aquatic Toxicity of Polar Narcotic Pollutants. Environmental Toxicology and Chemistry, Research Institute for Toxicology (RITOX), Utrecht University, Utrecht, The Netherlands.

Richardson, K.A., Wilmer, J.L., Smith-Simpson, D., and Skopek, T.R. (1986). Assessment of the genotoxic potential of unleaded gasoline and 2,2,4-trimethylpentane in human lymphoblasts in vitro. Toxicol Appl Pharmacol 82, 316-322.

Riddick, J.A., Bunger, W.B. and Sakano, T.K. (1986). Organic solvents. Wiley Interscience, New York.

Short, BG; Burnett, VL; Swenberg, JA. (1989) Elevated proliferation of proximal tubule cells and localization of accumulated alpha2u-globulin in F344 rats during chronic exposure to unleaded gasoline or 2,2,4-trimethylpentane. Toxicol Appl Pharmacol 101:414–431.

Spencer, P.S., Schaumburg, H.H., Sabri, M.I. and Veronesi, B. (1980). The enlarging view of hexacarbon neurotoxicity. CRC Crit Rev Toxicol. 7, 279-356.

Spencer, P.S. and Bischoff, M.C. (1987). Skin as a route of entry for neurotoxic substances. In: Dermatotoxicology, 3rd ed. pp.625-640. F.N. Marzulli and H.I Maibach, Eds. Hemisphere, Washington.

Shelby, M. and Witt, K. (1995). Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. Environ Mol Mutagen. 25, 302-313.

Shell Chemie (1975). Shell Industrie Chemicalien gids. Shell Nederland Chemie, Afd. Industrie-chemicalien, Wassenaarseweg 80, 's-Gravenhage, Nederland. 1.1.1975.

TNO, Division of Technology for Safety, Netherlands Organization for Applied Scientific Research (1986). Aquatic toxicity of compounds that may be carried by ships (MARPOL 1972, Annex II), Doc. # R 86/326a. TNO, The Netherlands.

Treon, J.F., Crutchfield, W.E. Jr. and Kitzmiller, K.V. (1943). The physiological response of animals to cyclohexane, methylcyclohexane and certain derivatives of these compounds II, inhalation. J of Indust Hyg Toxicol. 25, 323-347.

US EPA, (1999a). Determining the Adequacy of Existing Data. OPPT, EPA., Washington, DC, USA.

US EPA, (1999b). The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program. OPPT, EPA, Washington, DC, USA.

U.S. Environmental Protection Agency (U.S. EPA) (2000). EPI SuiteTM, Estimation Program Interface Suite, v3.12. U.S. EPA, Washington, DC, USA.

Van Wezel, A.P. and Opperhuizen, A. (1995). Narcosis due to environmental pollutants in aquatic organisms: residue-based toxicity, mechanisms, and membrane burdens. Crit Rev Toxicol. 25,255-279.

Verbruggen, E.M.J., Vaes, W.J.J., Parkerton, T.F., and Hermens, J.L.M.. (2000). Polyacrylate-coated SPME fibers as a tool to simulate body residues and target concentrations of complex organic mixtures for estimation of baseline toxicity. Environ Sci & Technol. 34,324-331

Verschueren, K. (1983). Handbook of Environmental Data on Organic Chemicals, Second Edition. Van Nostrand Rienhold, New York.

Yalkowsky, S. and Dannenfelser, R. (1992). Aquasol database of aqueous solubility. Version 5. College of Pharmacy, University of Arizona, AZ, USA.

Zepp, R. and Cline, D. (1977). Rates of direct photolysis in the aqueous environment. Environ Sci Technol. 11, 359-366.

Zobell, C.F. and Prokop, J.F. (1966). Microbial oxidation of mineral oils in Barataria Bay bottom deposits. Zeitschrift fürallgemeine Mikrobiologie, 6, 143-162.