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US High Production Volume Chemical Program

Category Summary

For

C5 Non-cyclics Category

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Prepared by:

Olefins Panel of the American Chemistry Council

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EXECUTIVE SUMMARY

The Olefins Panel of the American Chemistry Council (ACC) hereby submits the category summary report for the C5 Non-cyclics Category under the Environmental Protection Agency's High Production Volume (HPV) Chemical Challenge Program (Program). C5 refers to a 5 carbon (C) hydrocarbon molecule. The purpose of this report is to:

- Present results of an assessment that determined 10 production streams can be adequately characterized toxicologically and environmentally by applying previously existing data for selected stream components and newly developed data for streams in the C5 Non-cyclics Category HPV test program.
- Summarize the SIDS (Screening Information Data Set) endpoints that include physicochemical properties, environmental fate and effects, and human health impacts for the C5 Non-cyclics Category.
- Provide a description of manufacturing processes, potential exposure sources, and uses for all C5 Non-cyclics streams.

The C5 Non-cyclics Category contains 10 process streams:

- Pyrolysis C5s
- Hydrotreated C5s
- Pentenes
- Piperylene Concentrate
- Isoprene Concentrate
- Isoprene-Piperylene Concentrate
- Isoprene, High Purity
- Isoprene Purification Byproduct
- 2-Methyl-2-Butene (2M2B)
- Metathesis Byproduct

Formation of this category consisting of 10 production streams was considered feasible due to the:

- structural similarity between chemical constituents in the streams, and
- substantial overlap of stream compositions.

The resulting grouping was termed "C5 Non-cyclics Category". This report summarizes the HPV Program data and assessments for this category.

The 10 streams in the C5 Non-cyclics Category include 8 process streams that are complex mixtures of primarily C4 to C6 aliphatic hydrocarbons ranging from saturated to diolefinic molecules. The remaining 2 streams are high purity hydrocarbons, isoprene and 2-methyl-2-butene. Chemical Abstracts Service (CAS) registration numbers (RNs) used to represent the 8 complex streams (a mixture of chemicals arising from a chemical reaction or separation activity) may exhibit redundancies in that more than one CAS RN may correctly represent a single stream (depending on its source), and a CAS RN may be applicable to more than one stream.

Exposure

The major "parent" stream for this category is Pyrolysis C5s from which most of the other category streams are derived. Exposure potentials within the petroleum refinery industry (when the category streams are used for production of motor gasoline) were not included in this assessment. Specific use and exposure information for the 2M2B stream was not available. Specific use information for the Metathesis Byproduct stream was also not included because it was not produced in the year data were collected.

The category streams are either used (further processed) on site or transported in bulk to other industrial sites for processing. When transported, the category streams are moved in bulk quantities by barge, tank car, and tank truck.

The 6.4 billion pounds per year of category production is consumed as a chemical intermediate in other chemical manufacturing processes or used to produce gasoline. There are no expected direct consumer uses of the category streams. The major uses of the category streams are as intermediates to produce other streams in the category (30%), intermediates for the production of hydrocarbon resins or elastomers (18%), and feeds to motor gasoline production (50%).

Category streams are volatile liquids that have atmospheric boiling points in the range 80°F to 115°F. Inhalation is the most likely route of potential exposure, although dermal contact is also possible.

Category streams are produced, stored and transported in closed, pressurized systems and therefore there is minimal direct worker contact. Potential for exposure of workers at the olefins process units where the streams are produced and used occurs because of emissions from fugitive sources (equipment leaks) and from other emissions from the closed process. Emissions from these sources also present a potential for exposure to the environment and to areas bordering production facilities.

Since isoprene is ubiquitous in the environment, the general population is exposed to trace levels of this chemical through inhalation of ambient air. Exposure can also occur from handling consumer products or vegetation that contain this compound and by ingesting foods that contain isoprene. Ambient levels of isoprene have been reported to range to a level as high as 74 ppb in urban air down to 0.3 ppb in rural settings. Existing data for amylene (2M2B + other butenes) in ambient air indicate levels up to 12 ppb.

Neither the Occupational Safety and Health Administration (OSHA) nor ACGIH have established exposure limits for the streams in this category. The American Industrial Hygiene Association reports a Workplace Environmental Exposure Level for isoprene of 2 ppm, 8-hr TWA (time weighted average). OSHA has adopted PELs and/or ACGIH has adopted TLVs for some of the components found in the complex streams, including 1,3-butadiene, pentane (all isomers), cyclopentane, cyclopentadiene, 2,2-dimethylbutane, 2-methylpentane, 1-hexene, and benzene.

EPA and individual states have published a number of environmental requirements intended to limit emissions of volatile organic compounds, which include all of the category streams.

Human Health

All members of the C5 Non-cyclics Category are expected to have a low order of acute toxicity. This is based upon the fact that the components are hydrocarbons, many of which are saturated and thus chemically unreactive. Additionally, components that are unsaturated such as isoprene and 2-methyl-2-butene have been shown to have low orders of acute toxicity in animals by both the oral and inhalation routes of exposure. 2-Methyl-2-butene was shown to have a low order of acute toxicity by the dermal route of exposure.

Projections are based upon read-across as well as knowledge that components are primarily biologically unreactive hydrocarbons. All members of the C5 Non-cyclics Category would be expected, at most, to be mildly irritating to the skin and eyes. In addition, acute inhalation exposures to isoprene and 2-methyl-2-butene demonstrate high LC₅₀s (low toxicity), and since the diolefin isoprene is likely the most toxic of all components within the category, virtually all other streams containing little (typically <20%) or no isoprene are reasonably anticipated to exhibit acute toxicity below that for pure isoprene. Only the Isoprene Concentrate stream with up to 80% isoprene would approach the acute toxicity potential of the pure chemical. Repeated dose studies have been conducted on two major components (isoprene and 2-methyl-2-butene) and two complex streams (Pyrolysis C5s and Hydrotreated C5s) from the C5 Non-cyclics Category. It is isoprene that

has been studied most extensively. The subchronic inhalation toxicity studies demonstrate clear species differences between rats and mice in susceptibility to isoprene. In tests conducted in both rodent species over a period of 2 weeks, 13 weeks, and 26 weeks, there were no significant compound-related toxic effects noted in the rat at inhalation exposures up to 7,000 ppm. In contrast, mice exhibited increased mortality, body and organ weight, and various blood and microscopic organ changes in these studies. The NOAEL for isoprene is <438 ppm. In compliance with the HPV testing commitment for this category, 2-methyl-2-butene, the Pyrolysis C5s stream, and the Hydrotreated C5s stream were tested in 28-day inhalation studies in rats. The results were as follows:

- 2-Methyl-2-butene - Some general (blood, irritation, liver enlargement) effects were observed in this study, but were slight and most apparent in those animals exposed to the highest dose, i.e., 7,000 ppm. The NOAEL in this study was 580 ppm.
- Pyrolysis C5s stream - Slight changes were observed in the livers while male rats exhibited kidney lesions (hyaline droplets) known as "light hydrocarbon nephropathy." This finding is a male rat-specific phenomenon and has no relevance for human risk assessment. A NOAEL of 300 ppm was observed in this study.
- Hydrotreated C5s stream - Only mild clinical signs (lethargy and salivation) were observed plus evidence of "light hydrocarbon nephropathy", liver, and olfactory changes. The NOAEL for this study was 1,000 ppm.

Results from these studies demonstrate isoprene *per se* exhibits the highest acute and repeated dose toxicities, and that streams containing lesser amounts ($\leq 25\%$) of this component have reduced toxicities as exemplified by the higher NOELs/NOAELs for the Pyrolysis C5s and Hydrotreated C5s streams.

The mutagenic potential of streams in this category appears to be very low. This is due to the finding that none of the substances tested (isoprene, 2-methyl-2-butene, Hydrotreated C5s stream) were active in the sensitive Ames assay. In addition, although isoprene and 2-methyl-2-butene were active in *in vivo* mouse assays (sister chromatid exchange & micronucleus), only 2-methyl-2-butene showed genetic activity (very weak) in rats. The Hydrotreated C5s stream was inactive in the *in vivo* assay, supporting the view that genetic activity for this category is sporadic, of low potency, and appears to be associated primarily with the mouse. Based on these findings, members of this category are considered to have negligible to minimal mutagenic potential.

Isoprene is the only stream that has been subjected to a carcinogenicity assessment. Two studies show clear evidence of carcinogenic potential of isoprene in mice. Rats, on the other hand, exhibited no increases in the incidence of malignant tumors. These exposures were associated with increases in the rates of benign tumors. Although the carcinogenicity data for this category is limited, the minimal genotoxicities observed for category members as well as the fact that tumor findings for the likely most toxic stream (isoprene) were restricted to mice, the C5 Non-cyclics Category must be considered as having negligible to minimal human carcinogenic potential.

Reproductive organs of rats were found to be only marginally affected (testes) in 13- and 26-week isoprene repeated dose inhalation studies conducted in rats. Mice were shown to be more sensitive with effects seen in both genders. The NOAEL in rats was 220 ppm, and 70 ppm in mice. The developmental toxicity of isoprene was shown to be nil in rats. Mice exhibited both maternal and developmental (non-teratogenic) effects. The NOAEL for developmental toxicity from mouse data was determined to be <280 ppm.

OECD 422 combined repeated dose and reproduction/developmental rat toxicity studies on 2-methyl-2-butene, Pyrolysis C5s stream, and Hydrotreated C5s stream produced no evidence of reproductive or developmental toxicity.

Overall, the isoprene studies demonstrate a clear species difference between rats and mice with respect to both reproductive and developmental toxicity with the mouse being the more sensitive. Based on these findings and the fact that isoprene exhibited the greatest activity, the C5 Non-cyclics Category streams with low isoprene contents are considered as having minimal potential to produce reproductive and developmental effects.

This human health assessment for the C5 Non-cyclics Category has been based upon data that include considerable attention towards the most active component (isoprene) of the streams. This most active status was assigned based upon the fact that it is a diolefin, it is known to result in a metabolite that is highly mutagenic (a diepoxide), and that exhibits higher toxicity than other category member streams, particularly in the mouse. As the mixed streams Pyrolysis C5s and Hydrotreated C5s (with lesser amounts of isoprene but greater levels of other category components, monoolefins and saturates) were also tested, it is highly likely that the toxicities and environmental effects for the category are encompassed within the reported database and assessment.

Environment

Results of distribution modeling show that streams in the C5 Non-cyclics Category will partition primarily to the air compartment, with a negligible amount partitioning to water. Although constituents have a moderate degree of water solubility, wet deposition of category constituents is not likely to play a significant role in their atmospheric fate because they rapidly photodegrade. Volatilisation to the air will contribute to the rapid loss of category constituents from aqueous and terrestrial habitats. In the air, these constituents have the potential to rapidly degrade through indirect photolytic processes mediated primarily by hydroxyl radicals with calculated degradation half-lives that can range from 1.2 to 31.8 hours. Aqueous photolysis and hydrolysis will not contribute to the transformation of category constituents in aquatic environments because they are either poorly or not susceptible to these reactions.

Streams and pure chemicals in this category are subject to biodegradative processes. Overall, the streams from this category are expected to degrade rapidly in the environment from a combination of physical and biological processes and not persist.

Aquatic toxicity data exist for two different complex streams and two pure substances in this category. The two complex streams combined contain constituents shared by the remainder of the streams within this category, and therefore justifies their use to characterize the potential effects of the untested streams. This application of read-across data is further supported by data for two pure substances (also constituents of most of the streams in this category), which demonstrated effect values within or very similar to the range of values for the complex substances.

The aquatic toxicity data suggest that the streams in this category will exhibit a moderate order of toxicity. The effect values fall within a relatively narrow range for each of the species tested. Acute toxicity values for a fish and invertebrate range from 3.0 to 8.4 mg/L, while the toxicity values for an alga range from 10.1 to 18.4 mg/L, with NOEC values ranging from 1.7 to 13.1.

Most notably, because category constituents possess limited potential to accumulate in aqueous media, the toxicity of category streams towards aquatic species is considered a minimal risk factor in the overall safety assessment of this category.

Conclusion

The extensive body of data available for mammalian and environmental endpoints on category streams and representative constituents of substances in this category, are sufficient to fully characterize the potential toxicity of category members and demonstrate the integrity of the category, itself.

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MEMBER COMPANIES**

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* Companies that are part of the Olefins Panel, but do not produce products in the C5 Non-cyclics Category.

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1 CATEGORY DESCRIPTION AND JUSTIFICATION

1.1 Category Identification

For purposes of the US High Production Volume (HPV) Chemical Challenge Program (Program), the C5 Non-cyclics Category test plan submitted in September 2001 (Olefins Panel, HPV Implementation Task Group, 2001) included 10 production streams and 16 Chemical Abstracts Service (CAS) registration numbers (RNs) (Table 1). C5 refers to a 5 carbon (C) hydrocarbon molecule. The test plan identified existing data and data needed, based on an extensive technical review of the category, to adequately characterize the 10 streams for the HPV Program endpoints.

Table 1. Production Streams, CAS RNs¹, and CAS RN Names in the C5 Non-cyclics Category

Production Streams	CAS RN	CAS RN Name
Pyrolysis C5s	68476-55-1	Hydrocarbons, C5-rich
	68476-43-7	Hydrocarbons, C4-6, C5-rich
	68527-19-5	Hydrocarbons, C1-4, debutanizer fraction
	68603-00-9	Distillates, petroleum, thermal cracked naphtha and gas oil
	68956-55-8	Hydrocarbons, C5-unsatd.
Hydrotreated C5s	68602-79-9 ²	Distillates, petroleum, benzene unit hydrotreater dipentanizer overheads
	68410-97-9	Distillates, petroleum, light distillate hydrotreating process, low-boiling
	68603-00-9	Distillates, petroleum, thermal cracked naphtha and gas oil
Pentenenes	68476-55-1	Hydrocarbons, C5-rich
	68527-11-7	Alkenes, C5
	68603-03-2	Distillates, petroleum, thermal cracked naphtha and gas oil, extractive
Piperylene Concentrate	68477-35-0	Distillates, petroleum, C3-6, piperylene-rich
	64742-83-2	Naphtha, petroleum, light steam-cracked
Isoprene Concentrate	68514-39-6	Naphtha, petroleum, light steam-cracked, isoprene-rich
	68476-43-7	Hydrocarbons, C4-6, C5-rich
	78-79-5	1,3-Butadiene, 2-methyl-
Isoprene-Piperylene Concentrate	68514-39-6	Naphtha, petroleum, light steam-cracked, isoprene-rich
	68476-55-1	Hydrocarbons, C5-rich
Isoprene, High Purity	78-79-5	1,3-Butadiene, 2-methyl-
Isoprene Purification Byproduct	68606-36-0	Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product
	68476-55-1	Hydrocarbons, C5-rich
2-Methyl-2-Butene	513-35-9	2-Butene, 2-methyl-
Metathesis Byproduct	68606-29-1	Hydrocarbons, C4 and C8, butene concentrator by-product

¹ The CAS numbers associated with corresponding production streams are shown in the table above. The definitions found in the TSCA Chemical Substance Inventory for the CAS RNs in this category can be vague with respect to composition. Therefore, it is not uncommon to find that one CAS RN is correctly used to describe different streams (different compositions) or that two or more CAS RNs are used to describe one stream (similar composition). The

Olefins Industry or others may use these same CAS RNs to represent substances that may, in various degrees, be dissimilar to the category streams. CAS RNs, other than those shown in this table, may be used to describe these streams in future reporting.

- 2 This CAS RN was not included in the original list of CAS RNs sponsored in this category. It has been added to this category summary report because it is an additional CAS RN that is sometimes used to represent the indicated process stream.

After all data were evaluated, it was determined that the 10 streams could be considered a category. The following category report summarizes HPV Program data for this C5 Non-cyclics Category. The 10 streams in the C5 Non-cyclics Category include 8 process streams that are complex mixtures and 2 streams that contain relatively high purity hydrocarbons; all streams are composed predominantly of C5 hydrocarbons. These streams contain significant levels of olefins. The typical compositions of the streams in this category are shown in Table 2.

Table 2. Typical Stream Composition by Component (% weight) for the C5 Non-cyclics Category

Component	Pyrolysis C5s	Hydrotreated C5s	Pentenes	Piperylene Concentrate	Isoprene Concentrate	Isoprene - Piperylene Concentrate	Isoprene	Isoprene Purification Byproduct	2-Methyl-2-Butene	Metathesis Byproduct
	(% weight)									
1-Butene		2								
2-Butene (isomer mix)	0 - 1				1 - 20					3
1,3-Butadiene	0 - 3							0 - 1		
1,2-Butadiene					1.2					
2-Butyne (dimethyl-acetylene)	0 - 2				1 - 2					
Isoprene (2-methyl-1,3-butadiene)	9 - 25	2	2.4	0 - 6	14 - 80	20	99.7	1 - 12		
1,4-Pentadiene	1 - 6		1		0 - 4	3		1 - 10		
1,3-Pentadiene (isomer mix)	6 - 23	2		31 - 60	1 - 15	14				
3-Methyl-1,2-Butadiene					3.5					
1,3-Cyclopentadiene (CPD)	2 - 23	5		1 - 4	0 - 15	2		0 - 1		
Dicyclopentadiene (DCPD)	1 - 19			0 - 1	0.4					
Cyclopentene	1 - 11	15 - 20		8 - 20	0 - 10	4				
Cyclopentane	0 - 2	2		1 - 5	8 - 15	1				
Isopentene		15 - 20								
1-Pentene (amylene)	3 - 12		13.7		0 - 10	6		3 - 11		3
2-Pentene (isomer mix)	2 - 10		18	1 - 10	1 - 16	5		3 - 9		41
2-Methyl-2-Butene	1 - 5	11	4	5 - 15	0 - 9	3			93	
2-Methyl-1-Butene	1 - 8		17.5		1 - 16	6			6.7	

Table 2. Continued

Component	Pyrolysis C5s	Hydrotreated C5s	Pentenes	Piperylene Concentrate	Isoprene Concentrate	Isoprene - Piperylene Concentrate	Isoprene	Isoprene Purification Byproduct	2-Methyl-2-Butene	Metathesis Byproduct
	(% weight)									
3-Methyl-1-Butene (isoamylene)	0 - 12				0 - 1	1				
Pentane	4 - 30	15 - 20	31.7	0 - 5	0 - 26	16		0 - 10		
Methyl Butenes								3 - 21		
Isopentane (2-methylbutane)	3 - 29	15 - 25	12.3		21	16		50 - 70		
n-Pentene		10 - 15								
3-Methyl-1,4-Pentadiene 3-Methyl-1-Pentene	0 - 2			1.7						
Methylpentenes C6 Hydrocarbons	2 - 4		1	5 1 - 5	0 - 3					
1-Hexene	0 - 3									4
2-Hexene										15
3-Hexene										8
Hexenes		1		2						
Methyl-2-Pentenes										24
2,2-Dimethylbutane (neohexane)	0 - 1			2.7						
2-Methylpentane		5								
Methylpentanes				16						
Hexane		1		3.3						
Benzene	0 - 1	1		0.2						
Dimers of CPD with other C4 and C5 Dienes, excluding DCPD	0 - 2									

- Note 1: The balance of these streams is expected to be other hydrocarbons that have boiling points in the range of the listed components.
- Note 2: The listed ranges should not be considered absolute values. They are instead the approximate highs and lows of the reported values, and are expected to be typical limit values.
- Note 3: The definitions, found in the TSCA Chemical Substance Inventory, for the CAS RNs included in this group are very general and vague with respect to composition. It is not uncommon to find that the same CAS number is used to describe multiple streams or that two or more CAS numbers are used to describe a single stream. Multiple CAS RNs applying to the same streams arose as various companies supplied somewhat different compositional information when they registered similar substances.

The TSCA Chemical Substance Inventory definitions for the CAS RNs in this and in other categories from the Olefins Panel's HPV Program can be very general and vague with respect to composition. Consequently, the data matrix for this category was developed based on 10 compositionally differentiated process streams, rather than on the CAS RNs in this category.

The C5 Non-cyclics Category streams arise from production processes associated with ethylene manufacturing (see Appendix I for a description of the ethylene and associated processes). The C5 Non-cyclics Category was developed by grouping ethylene manufacturing streams that exhibit commonalities from both manufacturing process and compositional perspectives. Briefly, descriptions of the 10 process streams are:

1. Pyrolysis C5s (or C5 fraction) stream consists of a hydrocarbon distillate fraction separated from pyrolysis gasoline (the C5+ portion of the cracked gas in the ethylene process). The carbon number of the constituents from this stream is predominantly C5, but the stream also typically contains relatively low levels of the higher boiling C4 substances (e.g. 1,3-butadiene) as well as low levels of the more volatile C6 hydrocarbons. Benzene content is typically 0.25% and present in the distillate largely due to azeotropes of benzene with other hydrocarbon species in the complex mixture. The 1,3-butadiene content is typically 1%. The stream contains significant levels of olefins, diolefins and cyclics.
2. Hydrotreated C5s stream results from the hydrogenation of Pyrolysis C5s stream over catalyst. Typically the stream that is charged to the hydrogenation reactor is a broader boiling range stream than the C5 fraction. For example, a full range pyrolysis gasoline may be hydrotreated and the resulting product then fractionated to produce the Hydrotreated C5s as a distillate fraction. The hydrogenation process may be either a one-stage or two-stage process. The one-stage process is typically a liquid-phase process where the primary objective is to selectively convert diolefins to monoolefins. The two-stage process is typically a vapor-phase, more severe hydrogenation that converts monoolefins to paraffins. Typically, Hydrotreated C5s are subject only to one-stage hydrogenation because the product is intended for use in gasoline where the monoolefins are desired components. Similar to Pyrolysis C5s, Hydrotreated C5s have a carbon number distribution that is predominantly C5, and contain low levels of the higher boiling C4 substances as well as low levels of the more volatile C6 hydrocarbons. Benzene content is typically 1%. Unlike pyrolysis C5s, the diolefin content in the Hydrotreated C5s stream is very low.
3. Pentenenes stream results from the Pyrolysis C5s stream, which is typically fractionated into concentrates of the reactive diolefins: isoprene, piperylene (1,3-pentadiene), and cyclopentadiene (as dimer). As a first step in producing these concentrates, the lighter boiling fraction of the stream, i.e., the compounds that are more volatile than isoprene, are sometimes removed as a distillate. This distillate is designated as Pentenes or the Pentenes Cut. The Pentenes stream has a carbon number distribution that is predominantly C5, consisting in part of iso-pentane and n-pentane, and the more volatile pentenes such as 1-pentene, with about 1 to 3% isoprene. This stream can also contain the C4 compounds that are present in the Pyrolysis C5s stream at comparable concentrations, including 1,3-butadiene, although the data in Table 2 does not indicate that they are present. Alternately, Pentenes can be removed later in processing, for example by distillation of the Isoprene Concentrate.

4. Piperylene Concentrate (*cis*- and *trans*-1,3-pentadiene) stream is produced from Pyrolysis C5s by first "heat soaking" the stream in order to dimerize 1,3-cyclopentadiene (CPD). This is necessary because the boiling point of CPD is within 2.5 °F of that of *trans*-1,3 pentadiene. The heat soak produces a mixture of CPD dimer and codimers (DCPD Concentrate) that can be removed as a bottoms product from the balance of the Pyrolysis C5 stream. After removal of the DCPD Concentrate, what is left of the Pyrolysis C5s can be charged to a distillation column (the isoprene-piperylene splitter) to yield Piperylene Concentrate as a bottoms product. The carbon number for Piperylene Concentrate constituents is predominantly C5. A typical Piperylene Concentrate stream composition includes 60% piperylenes, 10% 2-methyl-2-butene, and about 0.2% benzene.
5. Isoprene Concentrate stream is also derived from the isoprene-piperylene splitter (as described for the Piperylene Concentrate stream) as a distillate. The carbon number for Isoprene Concentrate constituents is predominantly C5. A typical Isoprene Concentrate stream contains 40% isoprene with the balance largely iso- and n-pentane and C5 monolefins. Pentenes, as described for the Pentenes stream, may or may not have been removed in the distillation sequence, and this has the corresponding effect on the concentration of the lower boiling pentene and pentane components in the Isoprene Concentrate.
6. Isoprene-Piperylene Concentrate stream, the intermediate process stream charged to the isoprene-piperylene splitter (as described for the Piperylene Concentrate stream), is sometimes isolated as a product. This stream typically contains about 20% isoprene and 14% piperylenes.
7. Isoprene, High Purity stream (98+% isoprene) is produced by separation from Isoprene Concentrate stream. This is accomplished using an extractive distillation process.
8. Isoprene Purification Byproduct stream is produced from the Isoprene purification process. The carbon number of the stream's constituents is predominantly C5 and the composition is largely iso- and n-pentane, plus lesser amounts of pentenes and about 5% isoprene. The byproduct stream may also contain 1,3-butadiene at approximately 0.5%.
9. 2-Methyl-2-Butene stream is sometimes separated from a mixed C5 stream by first converting to an intermediate, then separating the intermediate from the mix by distillation, and then cracking the intermediate back to yield product 2-methyl-2-butene.
10. Metathesis Byproduct stream is derived from a Metathesis process which converts ethylene and/or butenes into propylene. This process produces a byproduct referred to as Metathesis Byproduct, which is removed from the propylene. The Metathesis Byproduct stream is a gasoline stream consisting primarily of C5 and C6 olefins.

1.2 Purity/Impurities/Additives

Antioxidants or polymerization inhibitors such as BHT (butylhydroxytoluene) (CAS RN 128-37-0) or TBC (*tert*-butylcatechol) (CAS RN 98-29-3) are typically added to category streams that contain significant concentrations of dienes, which include Pyrolysis C5s, Isoprene Concentrate, Piperylene Concentrate, Isoprene-Piperylene Concentrate, and High Purity Isoprene. Typical target concentrations for these additives range from 10 to 50 ppm.

1.3 Physico-Chemical Properties

The 10 streams in this category include 8 that are complex, containing many different hydrocarbons (Table 2) that can vary in composition not only between manufacturers but also for an individual manufacturer, depending on feedstock type and operating conditions. The 9 constituents listed in Tables 3 and 4 comprise significant proportions of these complex streams, and thus were selected as posing the greatest influence on the physico-chemical (PC) properties of these streams. Therefore, the data for these constituents in conjunction with measured data for 2 streams, Pyrolysis C5s and

Hydrotreated C5s, (Table 5) can be used to adequately characterize the five PC endpoints of substances in this category for the HPV Program. The 2 relatively pure streams, Isoprene Concentrate and 2-Methyl-2-Butene, are represented by the properties listed for isoprene and 2-methyl-2-butene, respectively.

Table 3. Summary of Calculated Physico-Chemical Properties for Selected Chemicals Contained by Streams in the C5 Non-cyclics Category

Chemical	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure (hPa@ 25°C)	Log P _{ow}	Water Solubility (mg/L)
<i>Cis</i> -Butene-2	-120.4	27.82	2.31 E3	2.09	652.7
<i>Cis</i> -Pentene-2	-107.1	53.97	6.76 E2	2.58	245.1
3-Methyl-1-Butene	-120.5	28.20	1.20 E3	2.59	242.7
1,4-Pentadiene	-109.8	42.12	9.79 E2	2.52	278.2
Isopentane	-119.0	30.18	9.17 E2	2.72	184.6
Isoprene	-118.9	34.95	7.35 E2	2.58	247.2
n-Pentane	-106.9	46.01	6.84 E2	2.80	159.7
2-Methyl-2-Butene	-116.2	46.92	6.24 E2	2.64	218.7
Cyclopentene	-93.2	65.86	5.06 E2	2.47	307.2

Calculated values derived by the EPIWIN program (EPIWIN, 1999)

Table 4. Summary of Measured Physico-Chemical Properties for Selected Chemicals Contained by Streams in the C5 Non-cyclics Category

Chemical	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure (hPa @ 25°C)	Log P _{ow}	Water Solubility (mg/L)
<i>Cis</i> -Butene-2	-105.5	0.8	2.33 E3	2.31	423.5
<i>Cis</i> -Pentene-2	-140.2	36.3	6.75 E2	Na	Na
3-Methyl-1-Butene	-168.5	20.1	1.20 E3	Na	Na
1,4-Pentadiene	-148.8	26.0	9.97 E2	2.48	300.9
Isopentane	-159.9	27.8	9.19 E2	Na	Na
Isoprene	-145.9	34.0	7.33 E2	2.42	338.6
n-Pentane	-129.7	36.0	6.85 E2	2.39	49.8
2-Methyl-2-Butene	-133.7	38.5	6.24 E2	2.67	206.1
Cyclopentene	-135.1	44.2	5.06 E2	Na	Na

Na not available

Measured values from the EPIWIN experimental database (EPIWIN, 1999)

Table 5. Measured Physico-Chemical Properties for the Pyrolysis C5s and Hydrotreated C5s Streams in the C5 Non-cyclics Category

Stream	Boiling Range (°C)	Vapor Pressure (hPa @ 25°C)	Log P _{ow} Range (@ 21°C)
Pyrolysis C5s (HLS, 2002a)	25.0 to 56.5	5.85 E2	3.19 to 3.25*
Hydrotreated C5s (HLS, 2002b)	23.5 to 52.0	8.23 E2	2.64 to 4.21**

HLS Huntingdon Life Sciences Ltd.

* @ 21.0°C

** @ 21.5°C

The following sections identify the values used to define the five PC endpoints of the 8 complex streams in this category based on the data listed in Tables 3 and 4. The physicochemical endpoints for the two high purity streams can be characterized by the data specifically for that chemical (i.e., the Isoprene, High Purity stream is characterized by the isoprene data). The measured data are considered the appropriate primary data set to characterize the melting point range of the high purity streams.

1.3.1 Melting Point (Range)

Based on calculated constituent values, the streams in this category can have a melting point range of -120.5 to -93.2°C. Based on measured constituent values, the streams in this category can have a melting point range of -168.5 to -105.5°C. The calculated data compare relatively well with the measured data. The measured data are considered the appropriate primary data set to characterize the melting point range of the complex category members.

1.3.2 Boiling Point (Range)

Based on calculated constituent values, the streams in this category can have a boiling point range of 27.82 to 65.86°C. Based on measured constituent values, the streams in this category can have a boiling point range of 0.8 to 44.2°C. The calculated data compare relatively well with the measured data. The measured data are consistent with process knowledge, and thus are considered the appropriate primary data set to characterize the boiling point range of the complex category members.

1.3.3 Vapor Pressure (Range)

Based on calculated constituent values, the streams in this category can have a vapor pressure range of 5.06 E2 to 2.31 E3 hPa at 25°C. Based on measured constituent values, the streams in this category can have a vapor pressure range of 5.06 E2 to 2.33 E3 hPa at 25°C. The calculated data compare favorably with the measured data. The measured data are consistent with process knowledge, and thus are considered the appropriate primary data set to characterize the vapor pressure range of the complex category members.

1.3.4 Octanol-Water Partition Coefficient (Log P_{ow}, Range)

Based on calculated constituent values, the streams in this category can have a log P_{ow} range of 2.09 to 2.80. Based on measured constituent values, the streams in this category can have a log P_{ow} range of 2.31 to 2.67. The calculated data compare favorably with the measured data. The measured data are considered the appropriate primary data set to characterize the log P_{ow} range of the complex category members.

1.3.5 Water Solubility (Range)

Based on calculated constituent values, the streams in this category can have a water solubility range of 159.7 to 652.7 mg/L. Based on measured constituent values, the streams in this category can have a water solubility range of 49.8 to 423.5 mg/L. The calculated data compare relatively well with the measured data. The measured data are considered the appropriate primary data set to characterize the water solubility range of the complex category members.

1.4 Category Justification

The C5 Non-cyclics Category was developed by grouping select ethylene manufacturing streams that exhibit commonality from manufacturing process and compositional perspectives. The manufacturing relatedness of the category streams is described in Appendix I. Compositionally, category streams are composed largely of C5 hydrocarbons, which are predominantly olefins (Table 2), but some can also contain significant proportions of saturated C5s. Each of the streams contain a number of the same constituents at varying levels, with pentadienes, pentenes, and pentanes as the predominant constituents. Selected members were included in this category because they were also expected to exhibit similar biological effects because of their largely comparable compositions.

The strategy to demonstrate that the members of this category could be considered together in order to assess their human and environmental health hazards and fate for purposes of the HPV Program was to develop and/or evaluate existing data for:

- two complex category streams, Pyrolysis C5s and Hydrotreated C5s, which between them contain a range of chemical constituents largely found in the remaining complex streams and from which most of the other streams are derived (except metathesis product), and
- two pure substances, isoprene (CAS RN 78-79-5) and 2-methyl-2-butene (2M2B) (CAS RN 13-35-9), which can also be found as components in most complex streams in this category.

Additionally, it was planned to use the data for the two pure substances to characterize the two high purity streams, Isoprene, High Purity (>99% isoprene) and 2-Methyl-2-Butene (approximately 93% 2M2B), the latter contains a small concentration of 2-methyl-1-butene, which is expected to exhibit effects and behave similar to 2M2B.

After evaluating the human and environmental health effects and fate data for the complex and pure substances, it was determined that the results for all endpoints other than biodegradation were sufficiently similar to consider the substances described in Table 1 a category. Similar results were obtained from the two tested complex streams. The differences in composition between these two streams did not result in widely differing or conflicting results. Consequently, data from the two tested complex streams provided needed information to conduct “read-across” to the untested complex streams. Data from the two pure substances will be used to characterize the two high purity streams as well as add to the weight of evidence for the category.

Although the biodegradability of the tested substances varied, select substances tested demonstrated a lag phase before degrading, suggesting that other substances in this category would exhibit similar activity. The remaining environmental fate endpoints will be similar across the category because the physicochemical properties for the chemical constituents are similar.

2 EXPOSURE AND USE

The C5 Non-cyclics Category contains 16 CAS RNs (Table 1) that are associated with the following 10 process streams:

- Pyrolysis C5s
- Hydrotreated C5s
- Pentenes

- Piperylene Concentrate
- Isoprene Concentrate
- Isoprene-Piperylene Concentrate
- Isoprene, High Purity
- Isoprene Purification Byproduct
- 2-Methyl-2-Butene
- Metathesis Byproduct

These streams are manufactured in ethylene production units (see Appendix I) and account for 100% of annual C5 Non-cyclics production in the US.

The following discussion on the use of and potential exposures to streams in the C5 Non-cyclics Category covers 10 Olefins Industry commercial hydrocarbon streams that have a carbon number that is predominantly C5¹. The category streams include two high purity products, isoprene and 2-methyl-2-butene, and 8 streams that are complex with variable compositions. Category streams are isolated intermediates that are stored in controlled on-site facilities, or isolated intermediates with controlled transport to a limited number of locations within the same company or to second parties that use the chemical as an intermediate under controlled conditions with long-established technology. The major applications for the category streams are as:

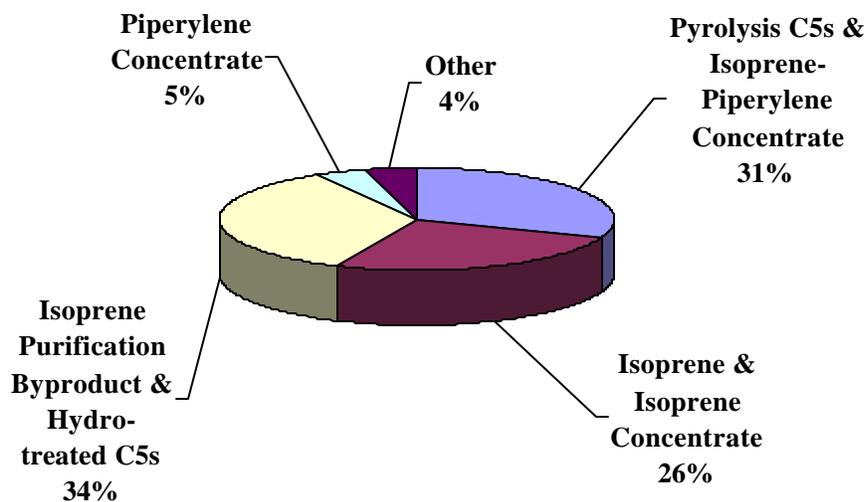
- intermediates to produce other streams in the category,
- intermediates to produce hydrocarbon resins or elastomers, and
- feedstocks for motor gasoline production.

The Pyrolysis C5s stream, which is derived from pyrolysis gasoline produced by the ethylene production process (see Appendix I), is the main source stream for members of this category. The stream identified as Metathesis Byproduct is an exception, and is derived from a process that converts ethylene or butenes to propylene.

There are 16 CAS numbers (Table 1) that are used by the Olefins Industry to represent the 10 category streams (Table 1). Distribution of the 6.4 billion² lbs/yr of category production among the category streams is shown in Figure 1. This assessment does not address potential exposures within the Petroleum Industry arising from the use of the category streams. When transferred to the Petroleum Industry, the volume of these streams represent only a small portion of similar streams managed by the Petroleum Industry.

¹ As produced, category streams contain predominantly C5s, however cyclopentadiene (a component of some of the streams) dimerizes at ambient storage conditions. Therefore, streams containing this monomer can be expected to also contain the dimer dicyclopentadiene.

² 6.4 billion lbs/yr is the total commercial production of C5 Non-cyclics Category streams reported by participants in this category, based on their 1998 TSCA IUR. Additional amounts of these streams may be produced by the Industry but not isolated.

Figure 1. C5 Non-cyclics Category Production by Stream

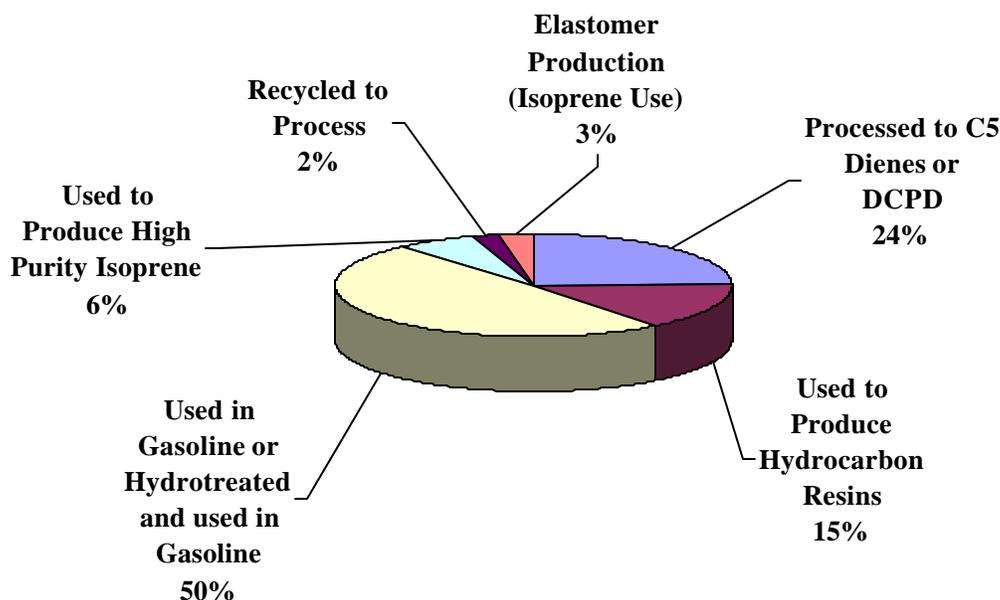
This screening level exposure assessment is based on information provided by six of the seven sponsors of this category, and from other available information.

Storage and Transportation of Category Streams

Category streams are either used on-site where they are produced, or shipped to other industrial sites for additional processing. When shipped between industrial sites, category streams are transported as liquids in closed systems by barge, tank car (rail), and tank truck (highway).

Uses

Available data on the use of the category streams are shown in Figure 2. Figure 2 does not include the 2-Methyl-2-Butene stream because specific use information was not available at the time of this assessment. One potential use of this stream is as a reactant in the production of hydrocarbon resins. Additionally, Figure 2 does not include specific data for the Metathesis Byproduct stream. That stream was not produced in the year that the use data was collected. Previous use of this stream was reported to be as a fuel. There are no expected direct consumer uses of the category streams.

Figure 2. Uses of the C5 Non-cyclics Category Streams

Routes of Potential Human Exposure

Category streams are volatile liquids that have atmospheric boiling points in a range of approximately 26.7°C (80°F) to 46.1°C (115°F). Inhalation is the most likely route of potential exposure. Exposure by dermal contact is also possible.

Sources of Potential Exposure

For workers in Olefins plants where streams in this category are manufactured and used, exposure is limited because the processes use closed systems. For industrial workers at these facilities, the most likely exposure potential occurs through inhalation of low-level concentrations in air of fugitive vapors, such as emissions from valves and pump seals; or during operations such as sampling, connecting and disconnecting bulk transportation vessels (tank cars and pressure barges), or during infrequent opening of equipment for maintenance.

These fugitive emissions and other emission sources also result in the potential for low-level ambient air concentrations of the HPV streams at locations neighboring the industrial facilities.

Since isoprene is ubiquitous in the environment, the general population is exposed to isoprene through inhalation of ambient air, by handling consumer products or vegetation that contain or emit this compound, and by ingesting foods that contain isoprene. Recent studies suggest that oak trees are the principal natural emitters of isoprene to the atmosphere. Isoprene generation exists extensively in nature, and is found in association with terpenes, camphors, diterpenes, vitamins A and K, chlorophyll, and other compounds isolated from animal and plant materials (HSDB, 2003).

Controls Limiting Exposure

Neither OSHA (Occupational Safety and Health Administration) nor ACGIH³ have established exposure limits for the streams in the Category.

³ Formerly known as the American Conference of Governmental Industrial Hygienists, now referred to only by the acronym.

The American Industrial Hygiene Association reports a Workplace Environmental Exposure Level (WEEL) for isoprene of 2 ppm, 8-hr TWA (time-weighted average) (HSDB, 2003).

OSHA has adopted PELs (Permissible Exposure Limits) and ACGIH has adopted TLVs (Threshold Limit Values) for some of the components found the complex streams, as shown in Table 6.

Table 6. Components present in some streams in the C5 Non-cyclics Category that have OSHA PELs or ACGIH TLVs

Component	OSHA PEL (ppm)	ACGIH TLV (ppm)	Component	OSHA PEL (ppm)	ACGIH TLV (ppm)
Cyclopentadiene	75	75	Pentane Isomers	1,000	600
Cyclopentane	-	600	Dicyclopentadiene	-	5

In addition to minimizing smog-creation due to hydrocarbon releases to ambient air, the release of the category streams from processing, storage, and transportation equipment at industrial facilities is avoided because these streams are highly flammable liquids.

Both the US (United States) EPA (Environmental Protection Agency) and state agencies enforce a wide range of volatile organic compound and hazardous air pollutant environmental regulations that control these emissions. The category streams contain volatile organic compounds (VOCs), and are therefore subject to US EPA and state environmental regulations that limit VOC emissions. The EPA new source performance standards, 40 CFR Part 60, may be applicable and limit emissions of VOCs at new or modified olefins process units where the streams in the category are produced and used. Subpart VV of 40 CFR Part 60 limits emission from equipment leaks, Subpart NNN limits emissions from distillation operations, and subpart RRR limits emissions from reactor systems. Facilities that produce and use the category streams are typically subject to state operating permits and state regulations that further limit VOC emissions.

Isoprene is designated as a hazardous substance under section 311(b)(2)(A) of the Federal Water Pollution Control Act, and is further regulated by the Clean Water Act Amendments of 1977 and 1978. These regulations apply to discharges of this substance. This designation includes any isomers and hydrates, as well as any solutions and mixtures containing this substance (HSDB, 2003).

Ambient Air Concentration Data (HSDB, 2003)

Isoprene was detected in five air samples collected in Houston, TX on April 2, 1974, at concentrations ranging from 5.2 to 74.3 ppb. Isoprene was detected at concentrations of 1.2 and 1.0 ppb in two air samples collected in Tulsa, OK suburbs and also detected at a concentration of 0.3 ppb in air samples collected in rural and downtown Tulsa, OK on July 27, 1978. Isoprene was detected at a mean concentration of 1.1 $\mu\text{g}/\text{m}^3$ (0.4 ppb) around the Los Angeles, CA area during a smog event on September 8 to 9, 1993. Amylene⁴ was detected at concentrations ranging from 3.7 to 11.5 ppb in ambient air samples collected around the Tulsa, OK area on July 27, 1978. Amylene was also detected in air samples collected from Rio Blanco County, CO at concentrations ranging from 0.9 to 3.0 ppb on July 24, 1978 and in air samples taken from the Smokey Mountains ranging from 0.1 to 3.7 ppb from September 25 to 26, 1978.

⁴The term "Amylene" as used in this reference appears to apply to 2-methyl-2-butene, or to complex mixtures of 2-methyl-2-butene with other butenes.

Ambient air concentrations (1994 through 1997) are available for isoprene and 2-methyl-2-butene for various industrial sites in Texas (TNRCC, 2003). Average concentrations for these two substances calculated from those data are shown in Table 7. The category streams account for only a portion of the measured concentrations, because these components are also emitted from other sources.

Table 7. Ambient Air Monitoring Data At Various Texas Industrial Sites (1994 through 1997)

Hydrocarbon	Average of Annual Mean Values (ppb)	Range of Annual Mean Values (ppb)	Average of Annual 24-hr High Values (ppb)	Range of Annual 24-hr High Values (ppb)
Isoprene	0.13	<0.01 – 0.67	0.81	<0.01 – 10.16
2-Methyl-2-Butene	0.27	<0.01 – 1.17	1.80	0.07 – 27.08

Estimates of Potentially Exposed Workers (HSDB, 2003)

NIOSH (National Institute for Occupational Safety and Health) (NOES Survey 1981 to 1983) has statistically estimated that 3,654 workers (578 of these are female) are potentially exposed to isoprene in the US. NIOSH (NOES Survey 1981 to 83) has statistically estimated that 67 workers are potentially exposed to 1,3-pentadiene in the US (1,3-pentadiene or piperylene is a component in some of the category streams). However, a number of limitations to this survey have been identified over the years, and the estimates of the number of workers potentially exposed to various substances are generally thought to be high.

Category Emissions

Emissions of the individual streams in this category or emissions of stream components are not included in the EPA Toxics Release Inventory (TRI)⁵. This inventory was established under the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA) and expanded by the Pollution Prevention Act of 1990.

Summary of Exposure Assessment

The HPV C5 Non-cyclics Category consists of two high purity streams, Isoprene High Purity and 2-Methyl-2-Butene, and 8 complex C5 streams. The major "parent" stream in the category is Pyrolysis C5s, from which most other category streams are derived. Exposure potentials within the petroleum refinery industry (when the category streams are used for production of motor gasoline) were not included in this assessment. Specific use and exposure information for the 2-Methyl-2-Butene stream was not included in this assessment because that information was not available. Specific use information for the Metathesis Byproduct stream was also not included because it was not produced in the year that data were collected.

The category streams are either used (further processed) on site or transported in bulk to other industrial sites for processing. When transported, the category streams are moved in bulk quantities by barge, tank car, and tank truck.

The 6.4 billion pounds per year of category production is consumed as a chemical intermediate in other chemical manufacturing processes or used to produce gasoline. There are no expected direct consumer uses of the category streams. The major uses of the category streams are as intermediates

⁵The EPA website for TRI is: <http://www.epa.gov/tri/>.

to produce other streams in the category (30%), intermediates for the production of hydrocarbon resins or elastomers (18%), and feedstocks for motor gasoline production (50%).

Category streams are volatile liquids that have atmospheric boiling points in the range 80°F to 115°F. Inhalation is the most likely route of potential exposure. Exposure by dermal contact is also possible.

Category streams are produced, stored and transported in closed, pressurized systems and therefore there is minimal direct worker contact with the streams. Potential for exposure of workers at the olefins process units where the category streams are produced and used occurs because of emissions from fugitive sources (equipment leaks) and from other emissions from the closed process. Emissions from these sources also present a potential for exposure to the environment and to areas bordering production facilities.

Since isoprene is ubiquitous in the environment, the general population is exposed to trace levels of this chemical through inhalation of ambient air. Exposure can also occur from handling consumer products or vegetation that contain this compound and by ingesting foods that contain isoprene. Recent studies suggest that oak trees are the principal natural emitters of isoprene to the atmosphere. Isoprene linkages exist extensively in nature; found in terpenes, camphors, diterpenes, vitamins A and K, chlorophyll, and other compounds isolated from animal and plant materials. Trace levels of isoprene are also exhaled in mammalian (including human) breath.

Neither OSHA nor ACGIH have established exposure limits for the streams in this category. The American Industrial Hygiene Association reports a WEEL for isoprene of 2 ppm, 8-hr TWA. OSHA has adopted PELs and/or ACGIH has adopted TLVs for some of the components found in the complex streams, including 1,3-butadiene, pentane (all isomers), cyclopentane, cyclopentadiene, 2,2-dimethylbutane, 2-methylpentane, 1-hexene, and benzene.

EPA and individual states have published a number of environmental requirements intended to limit emissions of VOCs, which include all of the category streams.

Isoprene concentrations of 5.2 to 74.3 ppb were reported in five air samples collected in Houston, Texas on April 2, 1974. Isoprene concentrations were reported as 1.2 and 1.0 ppb in Tulsa, Oklahoma suburbs, and 0.3 ppb in rural and downtown Tulsa on July 27, 1978. Ambient air concentration data for isoprene at various industrial sites in Texas from 1994 through 1997 indicate an average annual mean value of 0.13 ppb, with low and high values reported as <0.01 and 0.67 ppb. For 2-methyl-2-butene, the average of the annual mean values was 0.27 ppb, with low and high values reported as <0.01 and 1.17 ppb.

NIOSH estimates that 3,654 workers were potentially exposed to isoprene from 1981 to 1983 and 67 workers were potentially exposed to 1,3-pentadiene, a component of some of the category streams, during the same period.

3 ENVIRONMENTAL FATE

3.1 Photodegradation

The atmosphere is the primary environmental compartment of interest when considering fate processes that can impact the persistence of streams in the C5 Non-cyclics Category because they will partition to the air compartment when released to the environment. Results from an environmental distribution model support this assessment. The modelling results can be largely explained by the relatively high vapor pressure of the constituents evaluated. In spite of their water solubility, wet deposition of category constituents is not likely to play a significant role in their atmospheric fate. Constituents of streams in this category have the potential to degrade at a significant rate in the atmosphere through indirect photolytic process mediated primarily by

hydroxyl radicals (OH[•]). In comparison, direct photolysis is not expected to contribute to the degradative fate of these streams in the aqueous environment.

3.1.1 Direct Photodegradation

The direct photolysis of an organic molecule occurs when it absorbs sufficient light energy to result in a structural transformation (Harris, 1982a). The reaction process is initiated when light energy at a specific wavelength elevates a molecule to an electronically excited state. However, the excited state is competitive with various deactivation processes that can result in the return of the molecule to a non excited state.

The absorption of light in the ultra violet (UV)-visible range, 110-750 nm, can result in the electronic excitation of an organic molecule. Light in this range contains energy of the same order of magnitude as covalent bond dissociation energies (Harris, 1982a). Higher wavelengths (e.g. infrared) result only in vibrational and rotational transitions, which do not tend to produce structural changes to a molecule.

The stratospheric ozone layer prevents UV light of less than 290 nm from reaching the earth's surface. Therefore, only light at wavelengths between 290 and 750 nm can result in photochemical transformations in the environment (Harris, 1982a). Although the absorption of UV light in the 290-750 nm range is necessary, it may not be sufficient to induce some chemicals to undergo photochemical transformation. Energy may be re-emitted from an excited molecule by mechanisms other than chemical transformation, resulting in no change to the parent molecule.

A conservative approach to estimating a photochemical degradation rate is to assume that degradation will occur in proportion to the amount of light at wavelengths >290 nm absorbed by the molecule (Zepp and Cline, 1977). Saturated hydrocarbons do not absorb light above 200 nm. Characteristic absorbance maxima (λ_{max}) and associated molar absorptivities (ϵ) for three unsaturated hydrocarbons are listed in Table 8 (Harris, 1982a).

Table 8. Characteristic Absorbance Maxima (λ_{max}) and Associated Molar Absorptivities (ϵ) for Three Unsaturated Hydrocarbons

Hydrocarbon	λ below 290 nm	
	λ_{max}^*	ϵ
Ethylene	193	10,000
1,3-Butadiene	217	20,900
Benzene	255	215

* Values developed in organic solvents and regarded as approximate absorption maxima in aqueous solution.

Olefins with one double bond, two conjugated double bonds, or multiple un-conjugated bonds, which constitute the majority of the chemicals in the C5 Non-cyclics Category, do not absorb appreciable light energy above 290 nm. Streams in this category do not contain constituent molecules with significant potential to undergo direct photolysis. Therefore, this fate process will not contribute to a measurable degradative removal of chemical constituents in this category from the environment.

3.1.2 Indirect Photodegradation

In the environment, organic chemicals emitted into the troposphere are degraded by several important transformation processes. The dominant transformation process for most compounds is the daylight reaction with hydroxyl (OH[•]) radicals (Atkinson, 1988; Atkinson, 1989). The rate at

which an organic compound reacts with OH radicals is a direct measure of its atmospheric persistence (Meylan and Howard, 1993).

AOPWIN estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon an average atmospheric concentration of hydroxyl radicals.

Since the reactions necessary for this degradative process only take place in the presence of sunlight, the atmospheric half-lives are normalized for a 12-hour day. The nine chemicals selected to represent the atmospheric half-life range of streams in this category include one C4 and 8 C5 hydrocarbons that are predominant among the 16 CAS RNs (Table 9).

Table 9. Hydroxyl Radical Photodegradation Half-life of Selected Chemicals from Streams in the C5 Non-cyclics Category

Chemical	Calculated Half-life* (hrs)	OH [·] Rate Constant (cm ³ /molecule-sec)
<i>cis</i> -Butene-2	2.3	56.7 E-12
<i>cis</i> -Pentene-2	2.2	57.6 E-12
3-Methyl-1-Butene	4.5	28.6 E-12
1,4-Pentadiene	2.4	53.5 E-12
Isopentane	31.8	4.0 E-12
Isoprene	1.2	105.1 E-12
n-Pentane	31.7	4.0 E-12
2-Methyl-2-Butene	1.5	87.3 E-12
Cyclopentene	2.2	58.8 E-12

* Atmospheric half-life values are based on a 12-hr day and an OH[·] concentration of 1.5E6, which is the default concentration used by the model.

Atmospheric oxidation via hydroxyl radical attack can be a significant route of degradation for streams in this category. Based on calculated values, streams in this category can have an atmospheric half-life range of 1.2 to 31.8 hours as a result of indirect photolysis by hydroxyl radical attack.

3.2 Stability in Water (Hydrolysis)

Hydrolysis of an organic molecule occurs when a molecule (R-X) reacts with water (H₂O) to form a new carbon-oxygen bond after the carbon-X bond is cleaved (Gould, 1959; Harris, 1982b).

Mechanistically, this reaction is referred to as a nucleophilic substitution reaction, where X is the leaving group being replaced by the incoming nucleophilic oxygen from the water molecule. The leaving group, X, must be a molecule other than carbon because for hydrolysis to occur, the R-X bond cannot be a carbon-carbon bond.

The carbon atom lacks sufficient electronegativity to be a good leaving group and carbon-carbon bonds are too stable (high bond energy) to be cleaved by nucleophilic substitution. Under strongly acidic conditions the carbon-carbon double bond found in alkenes, such as those in the Fuel Oils Category, will react with water by an addition reaction mechanism (Gould, 1959). The reaction product is an alcohol. This reaction is not considered to be hydrolysis because the carbon-carbon linkage is not cleaved and because the reaction is freely reversible (Harris, 1982b). This reaction differs from other reactions with water such as hydration of carbonyls that can lead to the formation

of an alcohol beginning with the transfer of a proton from the water to an alkene. However, water by itself is too weak an acid to transfer a proton in the absence of a strong acid, which could effect such an acid catalysed electrophilic addition. Thus, hydrocarbons, including alkenes, are not subject to hydrolysis.

Chemicals 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 chemicals in this category are primarily olefins that contain at least one double bond (alkenes). The majority of the remaining chemicals are saturated hydrocarbons (alkanes). These two groups of chemicals contain only carbon and hydrogen. As such, their molecular structure is not subject to the hydrolytic mechanism described above. Therefore, chemicals in the C5 Non-cyclics Category streams have a very low potential to hydrolyze, and this degradative process will not contribute to their removal in the environment.

3.3 Distribution in the Environment

Fugacity-based multimedia modeling provides basic information on the relative distribution of a chemical between selected environmental compartments, which can include air, soil, water, sediment, suspended sediment, and biota. A widely used fugacity model, the EQC (Equilibrium Criterion) Level I model (Mackay *et al.*, 1996; Mackay, 1998) calculates chemical distribution between these compartments based on the input of basic physicochemical parameters including molecular weight, water solubility, log P_{ow} , and melting point.

Results of the EQC Level I model (Table 10) for selected chemical constituents of streams from this category suggest that they will partition primarily to air, with a small percentage partitioning to water. These results can be explained by their high vapor pressure. Distribution of these chemicals to each remaining compartment (soil, sediment, suspended sediment, biota) is calculated as less than 0.01%.

Table 10. Environmental Distribution as Calculated by the EQC Level I Fugacity Model for Selected Chemicals from Streams in the C5 Non-cyclics Category

Chemical	Distribution Per Environmental Compartment (%)					
	Air	Water	Soil	Sediment	Suspended Sediment	Biota
<i>cis</i> -Butene-2	99.98	0.02	<0.01	<0.01	<0.01	<0.01
<i>cis</i> -Pentene-2	99.97	0.03	<0.01	<0.01	<0.01	<0.01
3-Methyl-1-Butene	99.98	0.02	<0.01	<0.01	<0.01	<0.01
1,4-Pentadiene	99.97	0.02	<0.01	<0.01	<0.01	<0.01
Isopentane	99.98	0.01	<0.01	<0.01	<0.01	<0.01
Isoprene	99.96	0.03	<0.01	<0.01	<0.01	<0.01
<i>n</i> -Pentane	99.99	0.01	<0.01	<0.01	<0.01	<0.01
2-Methyl-2-Butene	99.97	0.02	<0.01	<0.01	<0.01	<0.01
Cyclopentene	99.94	0.04	<0.01	<0.01	<0.01	<0.01

Note: The distribution values were determined using physical property data from the EPIWIN (1999) database.

The nine chemicals selected to characterize the transport/distribution range include one C4 and 8 C5 hydrocarbons that are predominant across the streams in this category. Physical property data (Table 4) used in the model are from the EPIWIN (1999) database.

3.4 Biodegradation

Biodegradation is the use of an organic chemical by microorganisms as a source of energy and carbon. The parent chemical is broken down to simpler, smaller chemicals, which can eventually be converted to inorganic forms such as carbon dioxide, nitrate, sulfate, and water, depending on the composition of the parent chemical.

The microbial metabolism of aliphatic alkenes can be initiated by attack at the double bond (Watkinson and Morgan, 1990). Four degradative processes have been identified:

- Oxygenase attack upon a terminal methyl group to the corresponding alcohol, aldehyde, and acid
- Subterminal carbon oxygenase attack to the corresponding alcohol and ketone
- Oxidation across the double bond to the corresponding epoxide
- Oxidation across the double bond to the corresponding diol

Streams in the C5 Non-cyclics Category are composed predominantly of chemicals with a C5 carbon number. A smaller but significant percent of C4 hydrocarbons can also be present in select streams (Table 2).

Constituent chemicals from process streams in this category are hydrocarbons (Table 2) that are calculated to partition primarily to the air where physical processes will contribute to their rapid degradation (see Indirect Photodegradation above for specific degradation rates of selected chemicals from this category). Consequently, their availability to microbial degraders may be significantly limited in the environment. Because of the partitioning behavior of chemicals in this category, biodegradative processes may be less likely to contribute to their loss. However, streams from the C5 Non-cyclics Category do lend themselves to being evaluated for biodegradability because they are liquid at ambient temperatures.

Testing was conducted on two complex streams as well as two pure chemicals. The standard test guidelines applied included the OECD (Organization for Economic Co-operation and Development) 301D, Closed Bottle Biodegradation Test, and the OECD 301F, Manometric Respirometry Biodegradation Test. The two test methods use closed systems, which is necessary when evaluating volatile substances.

Data from the four studies were used to characterize the biodegradability of streams in this category (Table 11). With the exception of isoprene, the data suggest that streams in this category may biodegrade slowly during a 28-day test period, but there is potential for acclimation after which rapid degradation can occur. The 28-day results for two complex streams, Hydrotreated C5s and Pyrolysis C5s, and one pure substance, 2-methyl-2-butene, ranged from 7 to 11%. However, additional data for the Hydrotreated C5s stream suggest that although these substances may exhibit a significant lag phase, degradation to a relatively high extent can occur after microbial acclimation. The Hydrotreated C5s sample achieved 65% biodegradation on day 56 when an additional 28 days incubation period was allowed [this stream can contain 11% 2-methyl-2-butene and several chemicals found in the Pyrolysis C5s stream (Table 2)]. A distinct lag phase or acclimation period was also observed in the study that evaluated the biodegradability of isoprene. Although the lag phase was markedly shorter, approximately 9 days, once acclimated, the microbial consortium was able to degrade isoprene to an extent of 60% on day 18 of a 28-day study [isoprene can be a major constituent of the Pyrolysis C5s stream and is found at lower concentrations in the Hydrotreated C5s stream (Table 2)].

Table 11. Summary of Biodegradation Data for Selected Substances in the C5 Non-cyclics Category

CAS RN and Substance Name	Biodegradation (%)							
	Day 7	Day 9	Day 10	Day 11	Day 18	Day 28	Day 42	Day 56
68602-79-9 Hydrotreated C5s (EMBSI, 2004a)	3.1	2.7	2.6	2.1	0.6	11.2	54.6	65.4
68476-55-1 Pyrolysis C5s (HLS, 2003a)	5.0	-	-	1.0	8.0	7.0	-	-
78-79-5 Isoprene (EMBSI, 2004b)	4.5	3.8	17.9	33.1	60.1	60.9	-	-
513-35-9 2-Methyl-2-Butene (HLS, 2003b)	2.0	-	-	1.0	4.0	7.0	-	-

HLS Huntingdon Life Sciences Ltd.

EMBSI ExxonMobil Biomedical Sciences, Inc.

The results for the Hydrotreated C5s stream and isoprene illustrate the possible need for acclimation to occur before substances from this category begin to exhibit significant biodegradation. The data also suggest that these substances can be challenging to biodegrade and as a result, the acclimation period can vary.

As an example, the Hydrotreated C5s stream demonstrated little biodegradation during the first 22 days of the study after which the rate of biodegradation measured in the individual replicate (3) test systems markedly increased. However, that increase initiated on widely different days for each of the replicates; days 23, 27, and 31. By day 56 (twice the length of a standard test duration), the three replicates had achieved 38, 69, and 62% biodegradation. The difference between the replicates on day 56 was additional evidence that this sample presented a "challenge" to the degrading microorganisms, but after sufficient time, degradation occurred at rapid rates.

Differences in replicate biodegradation as was demonstrated in the study for the Hydrotreated C5s stream can occur when differing microbial consortia grow from the original microbial population added to the replicates. Although it could be expected that similar microbial populations between replicates would grow similarly, this may not always occur, and for substances that can be challenging to degrade, the difference in populations may be significant. In comparison, substances that are easily degraded, the positive control (sodium benzoate) for example, will not typically demonstrate a lag phase or acclimation period. Biodegradation can occur at a rapid rate in less than a day, and if a lag phase is demonstrated, it is typically less than 3 days. Additionally, biodegradation rates and extents of biodegradation in the replicates tends to be similar with relatively small standard deviations demonstrated by the biodegradation extent data.

A similar pattern of degradation was also demonstrated in the isoprene study. Although the acclimation phase was shorter in duration, the replicate variability as to when degradation became significant and at test termination, day 28, was similar to what was seen in the Hydrotreated C5s stream results. The replicates in the isoprene study achieved an extent of biodegradation equal to 55, 53, and 75%. The data from these two substances suggest that category members can exhibit high extents of biodegradation, but rapid rates of degradation will occur only after the microbial

population has an opportunity to acclimate, and that acclimation periods will vary between microbial populations.

The results for the Hydrotreated C5s stream and isoprene appear to illustrate the need for acclimation to occur before these substances can exhibit a relatively rapid rate of biodegradation. The data also suggest that these substances can be challenging to biodegrade.

The lack of biodegradation in selected studies during the 28-day exposure is not believed to result from toxic effects. The four substances showed no inhibitory affect on the normal degradative activity of the microbial inocula used to evaluate their biodegradability (Huntingdon Life Sciences Ltd; 2003a, 2003b, 2003c, 2003d).

3.5 Abiotic and Biotic Degradation Summary

The stream constituents from the C5 Non-cyclics Category will partition primarily to the air where physical degradative processes will dominate their fate. Data show that these chemicals are subject to rapid physical degradation in the air from hydroxyl radical attack. Constituent chemicals from category streams are not subject to direct photolytic processes or hydrolysis. Selected streams and pure chemicals that represent streams in this category are subject to biodegradative processes. Streams and constituents in this category exhibited a wide range of biodegradability under standard testing procedures. However, the data suggest that category streams can exhibit high extents of biodegradation and that once acclimation occurs, rapid rates of biodegradation are possible. Overall, the streams from this category are expected to degrade rapidly in the environment from a combination of physical and biological processes.

4 HUMAN HEALTH HAZARDS

4.1 Effects on Human Health

4.1.1 Acute Toxicity

Studies in Animals

Isoprene

Oral

An acute oral toxicity study was conducted in Wistar rats. In this study, 15 animals per sex were administered isoprene in oil. The oral LD₅₀ was determined to be 2043 to 2210 mg/kg. No other details were provided (Kimmerle and Solm ecke, 1972).

Inhalation

An acute inhalation exposure study was conducted by Shugaev (1969) to determine the concentrations of hydrocarbons such as isoprene in various tissues at lethal exposure concentrations. In this pre-GLP study minimal detail is provided. The rats were exposed for four hours and the mice for two hours. The LC₅₀ values reported in this study are as follows:

- Rat LC₅₀ (4 hour) = 180 mg/L or 64,620 ppm (confidence limits 130 to 181 mg/L; p ≤ 0.05)
- Mouse LC₅₀ (2 hour) = 157 mg/L or 56,363 ppm (confidence limits 129 to 252 mg/L; p ≤ 0.05)

2-Methyl-2-Butene

Oral

In order to determine the acute oral LD₅₀ of 2-methyl-2-butene (2M2B) two studies were performed: a range finding study in which two albino Wistar rats of each sex were dosed with 0.5, 1.0, and 5.0 ml/kg, and a second study in which six albino Wistar rats of each sex were dosed. In both studies the dosing was by intraesophageal intubation using a ballpoint needle fitted to a syringe. Because the test material was so volatile, it was necessary to keep it on ice until it was dosed. Therefore, the animals received 2M2B at a temperature of approximately 24.5°C. After dosing the animals were given food and water *ad libitum* and observed for toxicological signs over the following 14 days. Body weights were recorded at 7 and 14 days. Based on the results of the range-finding study, the acute oral LD₅₀ value was estimated to be between 1 and 5 ml/kg. To determine a more accurate LD₅₀ value, groups of six males and six females were dosed with 1.0, 1.6, 2.5, 4.0, 6.3, and 10 ml/kg. In this study, the majority of deaths occurred within the first 3 days following dosing with most survivors recovering from signs of intoxication by the third day. All but one of the survivors had gained weight by the conclusion of the 14-day observation period. The oral LD₅₀ was estimated to be in the range of 1 to 4 ml/kg (i.e., 700 to 2600 mg/kg) (Dewar, 1980).

Dermal

The acute (24 hour) dermal toxicity of 2M2B was determined using a method based on that of Noakes and Sanderson (1969). Two tests were performed, a range finding test in which two albino Wistar rats of each sex were dosed with 0.5, 1.0, and 2.0 ml/kg and a second test in which six rats of each sex were dosed with 3.03 ml/kg. The calculated dose was applied to the shaven skin by syringe, the dose being altered by varying the volume of the material applied. It was necessary to apply the material at a temperature of approximately 5°C on account of its volatility. The test material was covered with aluminum foil held in place by a double overwrap of waterproof adhesive tape. The rats were individually housed for the next 24 hours, food being withheld but water given *ad libitum*. At the end of the 24 hours exposure period, the foil and dressing were removed and the skin washed with warm dilute detergent solution and then dried. The animals were returned to group housing and observed for signs of toxicity over the following 14 days.

Initial 7 day and 14 day body weights were recorded. In the range finding study, the acute dermal LD₅₀ was estimated to be >2.0 ml/kg. In order to obtain a more accurate LD₅₀ value, a group of six males and six females were dosed with 3.03 ml/kg, which is equivalent to a dose of 2 g/kg. No mortalities were recorded and there were no signs of systemic toxicity. All animals gained weight within one week of dosing. Thus, the acute dermal LD₅₀ value of 2M2B is >2 g/kg (Dewar, 1980).

Inhalation

In this study, groups of 5 male and 5 female albino Wistar rats were exposed for 4 hours to a test atmosphere containing 6.1 per cent (v/v) 2M2B. During the exposure the animals became narcotized, but revived within 30 minutes of cessation of exposure. There were no deaths and macroscopic and microscopic examinations at necropsy of animals killed 14 days post-exposure revealed no compound related effects. The acute 4 hours inhalation LC₅₀ of 2M2B in rats is greater than 6.1% (i.e., 61,000 ppm) (Blair, *et al*, 1982).

Conclusion

Isoprene and 2M2B have a low order of acute toxicity in animals by the oral and inhalation routes of exposure (Table 12). 2M2B has also been shown to have a low order of acute toxicity by the dermal route of exposure.

Table 12. Summary of Acute Inhalation Toxicity Data for the C5 Non-cyclics Category

CAS RN and Substance Name	Test Organism	Exposure Duration (hours)	LC ₅₀ (ppm)
78-79-5 Isoprene	Rat	4	64,620
78-79-5 Isoprene	Mouse	2	56,363
513-35-9 2-Methyl-2-Butene	Rat	4	>61,000

4.1.2 Irritation

Studies in Animals

Isoprene

Skin Irritation

A skin irritation study was conducted in two New Zealand White rabbits (Kimmerle and Solmecke, 1972). In this study, the skin of the rabbit ears was painted twice per day for 5 consecutive days with 100% isoprene. Reversible erythema was observed. However, the severity of this erythema was not noted.

Respiratory Tract Irritation

A repeated dose inhalation study conducted in mice and rats exposed up to 7,000 ppm isoprene for 6 hours/day, 5 days/week for 13 weeks showed no gross microscopic lesions in the respiratory tract of rats and female mice (Melnick *et al.*, 1994). In male mice degeneration of the olfactory epithelium was observed at 7,000 ppm, but not at lower concentrations. The NOAEL in this study was 2,200 ppm.

2-Methyl-2-Butene

Skin Irritation

An occlusive patch test based on the method of Draize (1975) was used to assess the primary skin irritation induced by 2M2B applied neat. Three male and three female New Zealand White rabbits were used. After 24 hours exposure, the intact and abraded test sites were examined and scored for erythema and edema on a graded scale (0 to 4) at 24, 48, and 72 hours and 7 days post-dosing. The primary irritation score calculated according to the method of Draize was 1.79. On the basis of this score 2M2B may be regarded as being mildly irritating to rabbit skin. The erythema and edema scores at 7 days were higher than those at 72 hours. However, at 7 days all the skin patches were beginning to dry out and flake, and it is possible that a contributory factor to the slightly higher erythema scores was the animals scratching these areas (Dewar, 1980).

Eye Irritation

The method of Draize (1963) was used to assess the eye irritancy of 2M2B. Six New Zealand white rabbits were used. The reactions of the animals were observed immediately after instillation. A visual assessment of eye irritancy was made at 1 hour, 1 day, 2 days, 3 days, and 7 days after instillation or until the irritancy was no longer discernible. The mean total scores for the responses of the conjunctiva, cornea and iris at 1 hour, 1, 2, 3, and 7 days were 0.5, 0, 0, 0, and 0,

respectively. Based on these results, 2M2B should be considered as non-irritating to rabbit eyes (Dewar, 1980).

Conclusion

Based on the available data, 2M2B should be considered as mildly irritating to skin and non-irritating to eyes. The respiratory tract irritation potential of 2M2B is not known, as no data are available for this endpoint.

4.1.3 Repeated Dose Toxicity

Studies in Animals

Isoprene

Inhalation

A 2-week repeated dose inhalation study was conducted in mice and rats (Melnick *et al.*, 1990). In this study, F344 rats and B6C3F1 mice (20 animals/sex/group/species) were exposed by inhalation to isoprene at concentrations of 0; 438; 875; 1,750; 3,500; or 7,000 ppm for 6 hours/day, 5 days/week for two weeks. Ten animals/sex/group/species were used for clinical pathology evaluations after 4 exposures in rats or 5 exposures in mice. The remaining 10 animals per group were used for histopathology at the end of the study. Body weights and clinical observations were recorded weekly. Necropsies were performed and major tissues/organs preserved. Histopathologic examinations were performed on the control and high exposure animals (7,000 ppm) plus all lower dose groups until an apparent no-observed-effect level was found.

In rats, there were no treatment-related changes in survival, body weight gain, clinical signs, hematologic or clinical chemistry parameters, organ weights, or incidences of gross or microscopic lesions. In the B6C3F1 mice, decreased body weight gain was observed only in males in the 7,000 ppm exposure group. Other treatment related effects observed in mice exposed to isoprene included: a) slight increases in liver weight, b) decreases in thymus, spleen, and testis weights, c) hematologic changes such as reductions in red blood cell counts, hemoglobin concentrations, and volume of packed red cells, and d) microscopic lesions which included thymus and testicular atrophy, olfactory epithelial degeneration, and forestomach epithelial hyperplasia. The decreased spleen weights in mice exposed to isoprene were not associated with histopathological alterations in this organ.

This study demonstrated that there is a clear species difference in the susceptibility of rats and mice isoprene exposure. In rats, there were no observable toxicological effects at any dose following the 2-week exposure. However, in mice, exposure to isoprene for 2 weeks induced changes in hematological parameters, body and organ weights and produced microscopic lesions in certain tissues at levels as low as 438 ppm.

A 13-week repeated dose inhalation study was conducted in mice and rats (Melnick *et al.*, 1994). In this study, F344 rats (10/sex/group) and B6C3F1 mice (10/sex/group) were exposed to 0; 70; 220; 700; 2,200; and 7,000 ppm isoprene, 6 hours/day, 5 days/week for 13 weeks. Body weights and clinical observations were recorded weekly. Blood samples were collected for clinical pathology evaluations on days 4, 24, and at the end of the study. Urine samples were collected from rats during week 12. After thirteen weeks of exposures, all rats and mice were sacrificed and evaluated histopathologically. Organ weights were also recorded.

In rats, there were no exposure-related effects observed for survival, body weight gain, clinical signs of toxicity, hematology or clinical chemistry parameters, urinalysis, organ weights, or the incidence of gross or microscopic lesions. In mice, there were no effects on survival, body weight

gain, or clinical signs of toxicity. However, the male and female mice exposed to 700 ppm and higher showed hematologic effects indicative of a nonresponsive, macrocytic anemia at day 24 and after thirteen weeks. Focal epithelial hyperplasia of the forestomach was also observed in both males and females. Degeneration of the olfactory epithelium and cytoplasmic degeneration of the liver were also observed in male mice at the highest concentration (i.e., 7,000 ppm). The male mice exposed to 7,000 ppm exhibited testicular weights reduced 35% compared to the controls.

In conclusion, no toxicological effects were evident in rats exposed up to 7,000 ppm for 13 weeks. However, in mice, hematological and histopathological changes were observed at exposures of 700 ppm and higher. This 13-week repeated dose inhalation study, conducted as part of a 26-week carcinogenicity study, confirmed the species difference between rats and mice in susceptibility to isoprene.

A 26-week repeated dose inhalation exposure study was conducted with isoprene in F344 rats and B6C3F1 mice (Melnick *et al.*, 1994). In this study, groups of 40 male B6C3F1 mice and Fischer 344 rats were exposed to 0; 70; 220; 700; 2,200; or 7,000 ppm isoprene vapor by inhalation for 6 hours/day, 5 days/week for 6 months. At the end of the 26-week exposure period, 10 rats and 10 mice/group were sacrificed and evaluated. The remaining animals were allowed to recover for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Body weights and clinical observations were recorded weekly throughout the study. Blood samples were collected for clinical pathology evaluations after 26 weeks exposure. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically. Organ weights were recorded at both intervals. Twenty mice/group were evaluated for forelimb and hindlimb grip strength after 26 weeks exposure; 10 mice/group were also evaluated at 2 days, 1-, 3- and 6-months post-exposure.

After 26 weeks exposure, rats exhibited no treatment-related mortalities, bodyweight change nor laboratory findings. The only treatment-related effect was an increased incidence and severity of interstitial cell hyperplasia of the testis at 7,000 ppm (10/10, mild severity) compared with controls (1/10; minimal severity); this lesion was seen in all recovery groups (28/30-30/30 animals per group at 70 - 7,000 ppm), but also occurred at a high incidence in controls (25/30), and there was no concentration-related trend.

In mice, there was reduced survival at 7,000 ppm from approximately 18 weeks onwards. Early mortality was attributed to neoplastic lesions as well as sacrifice of animals showing hindlimb paralysis towards the end of the exposure period, primarily at 7,000 ppm. Similar hematological changes occurred after 26 weeks as were seen after 13 weeks exposure. Hindlimb grip strength was statistically significantly reduced at 220 ppm and above, up to approximately 4 weeks post-exposure. Statistically significantly increased incidences of testicular atrophy (5/10), degeneration of olfactory epithelium (10/10) and minimal degeneration of the spinal cord white matter (10/10) were seen at 7,000 ppm after 26 weeks exposure. In recovery groups, these latter lesions were seen with statistically significantly increased incidences in the lower exposure concentration groups also. Degeneration of olfactory epithelium in recovery groups occurred at 220 ppm and above (5/29 at 220 ppm, 28/28 at 7,000 ppm) compared with control (1/30). The incidence of spinal cord degeneration was increased at 70 ppm and above (20/30 at 70 ppm, 13/28 at 7,000 ppm, 4/30 in controls); the incidence of testicular atrophy in recovery exposure groups was not statistically significantly different from controls.

A chronic study was conducted in B6C3F₁ mice. In this study, mice were exposed to isoprene by inhalation for either 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks (Placke *et al.*, 1996). Twelve groups of 50 male B6C3F₁ mice were exposed to 0, 10, 70, 140, 280, 700 or 2,200 ppm of isoprene vapor for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period, leading to a total planned study length of 104 weeks. Female mice (50/group) were exposed to 0, 10, and 70 ppm of isoprene, 8 hours/day for 80 weeks and also held for observation through

week 104. Selected groups of mice were removed at the end of 20 or 40 weeks of exposure, and were held in holding chambers for the duration of the 80 week test period. At the end of 80 weeks, all surviving animals were moved to a holding room through study week 104 and then necropsied beginning in study week 105.

Survival rate was reduced to less than 50% of control, from week 80 onwards at 280 ppm and above; animals in these groups were necropsied at week 96. No clinical signs of toxicity were seen other than those associated with tumor development. Bodyweight and hematological parameters were unaffected by treatment. At necropsy, treatment-related gross lesions observed were opacity of the eyes, often with protrusion due to Harderian gland enlargement. Nodules and masses in the forestomach mucosa, liver and lung; enlargement of the spleen and mesenteric lymph node; and reduction in size and weight of testis were also observed. Effects were apparent at 280 ppm and above, but the dose levels at which particular effects were seen were not clearly stated. In females, there was a reduction in ovarian weight at both exposure concentrations, 10 and 70 ppm, which did not reach statistical significance, and which may have been treatment-related. A slightly increased incidence of hyperplasia of the alveolar lining and of the forestomach mucosa were seen at higher doses in males; there was an increased incidence of mild metaplasia of focal areas of the olfactory epithelium down to the respiratory epithelium in males apparently at 2,200 ppm and in females at 70 ppm. No other clear treatment-related non-neoplastic changes were observed. Additional details about tumors that occurred in this study are given in the carcinogenicity section.

A chronic inhalation study was also conducted in rats. In this study, groups of 50 male and female F344/N rats were exposed to 220, 700, or 7,000 ppm isoprene by inhalation, 6 hours per day, 5 days per week, for 104 weeks (NTP, 1999). The survival of exposed males and females was similar to that of the chamber controls. Mean body weights of exposed male and female rats were also similar to those of the chamber controls throughout the study.

Non-neoplastic effects were observed in male rats only. They included renal tubule hyperplasia and splenic fibrosis. The incidence of renal tubule hyperplasia was as follows: 0/50, 2/50, 6/50, and 8/50, respectively, in the standard evaluation. When standard and extended evaluations were combined, the incidences were: 7/50, 6/50, 13/50, and 18/50, respectively.

The incidence of splenic fibrosis was as follows: 11/50, 14/50, 24/50, and 22/50, respectively.

No other clear treatment-related non-neoplastic changes were observed. Additional details about tumors that occurred in this study are given in the carcinogenicity section.

2-Methyl-2-Butene

Inhalation

An OECD 422 combined general toxicity and reproduction/developmental toxicity screening study was conducted in Sprague-Dawley rats (Huntingdon Life Sciences Ltd, 2003e). In this study, groups of 12 male and 12 female rats were exposed by inhalation to 0; 580; 2,000; or 7,000 ppm (approximately 1,660; 5,720; or 20,000 mg/m³) 2M2B for approximately 6 hours/day. In the main study, i.e., repeated-dose general toxicity study, the males and females were exposed for 28 days, respectively. Parameters measured during this study included clinical signs, a detailed functional observational battery, motor activity, bodyweight, food consumption, hematology, blood chemistry, organ weight, and macroscopic and microscopic pathology.

The clinical signs observed during this study included half-closed eyes on day 1 in the groups exposed to 2,000 and 7,000 ppm. In addition, these animals exhibited a lower level of response to external stimuli. This latter finding was observed on one other occasion in the high dose animals. No signs were observed indicative of any general systemic effects either during routine clinical

examination or during the functional observational battery. There was a slightly lower bodyweight gain at 7,000 ppm, and slightly longer clotting times at 2,000 ppm (prothrombin time for females) and 7,000 ppm (prothrombin times for both sexes and activated partial thromboplastin times for males). Cholesterol levels were increased among the females exposed to 7,000 ppm but in the absence of any further effects in the clinical chemistry parameters or the males this finding is of doubtful significance.

Pathological changes were noted among the high dose female rats in the liver, as evidenced by increased organ weight and minimal centrilobular hepatocyte hypertrophy. There was also a decreased incidence of extramedullary hematopoiesis in the spleen in the high dose animals, and an increase in goblet cell hyperplasia in the nasal passages of the high dose males. In addition, a slight increase in severity of myocardial inflammatory heart lesions and in cortical/medullary tubular basophilia in the kidneys was observed in the high and intermediate dose males.

Although some general systemic effects were observed in this study, these effects were slight and most apparent in animals exposed to the highest dose, 7,000 ppm, and to a lesser extent to those exposed to 2,000 ppm. Based on these observations, the No Observed Effect Level in this study was 580 ppm.

Pyrolysis C5

Inhalation

An OECD 422 combined general toxicity and reproduction/developmental toxicity screening study was conducted in Sprague-Dawley rats (Huntingdon Life Sciences Ltd, 2003f). In this study, groups of 12 male and 12 female rats were exposed by inhalation to 0; 100; 300; or 1,000 ppm Pyrolysis C5s stream for approximately 6 hours/day. In the main study, i.e., repeated-dose general toxicity study, the males and females were exposed for 28 days, respectively. Parameters measured during this study included clinical signs, a detailed functional observational battery, motor activity, bodyweight, food consumption, hematology, blood chemistry, organ weight, and macroscopic and microscopic pathology.

No signs consistent with a general systemic effect were observed during the routine clinical examination or during the functional observational battery. Inter-group differences in hematological and biochemical parameters were not considered to be treatment related.

The observed histopathological changes were restricted to the liver (minimal centrilobular hepatocyte hypertrophy) of the high dose rats, associated with slightly elevated liver weights. These effects were considered to be adaptive, not adverse. However, the male rats also exhibited a higher kidney weight and incidence of cortical tubules with hyaline droplets apparent in all treatment groups with other associated kidney lesions. No pathological changes were apparent in the kidneys of treated females. The reported effects on kidneys are considered a male rat-specific phenomenon and have no relevance for human risk assessment.

Due to the histopathological changes in the male kidneys a no effect level for general systemic toxicity was not established. However, if one considers only those effects considered potentially relevant to humans, a NOAEL of 1,000 ppm was established for male rats. In females, a NOEL of 1,000 ppm was established.

Hydrotreated C5s

Inhalation

An OECD 422 combined general toxicity and reproduction/developmental toxicity screening study was conducted in Sprague-Dawley rats (Huntingdon Life Sciences Ltd, 2003g). In this study,

groups of twelve male and twelve female rats were exposed by inhalation to target concentrations of 0; 1,000; 3,000; or 8,500 ppm Hydrotreated C5s. In the main study, i.e., repeated-dose general toxicity study, the males and females were exposed for 6 hours/day for a period of 4 weeks. Parameters measured during this study included clinical signs, a detailed functional observational battery, motor activity, bodyweight, food consumption, hematology, blood chemistry, organ weight and macroscopic and microscopic pathology. The study mean analysed concentrations of Hydrotreated C5s over the duration of the study were 992; 3,033; or 8,502 ppm, respectively. These levels were in good agreement with the target exposure levels.

Clinical signs observed included salivation in the high dose animals, i.e., 8,502 ppm, during a portion of the treatment period, and on one occasion, animals were noted as being lethargic. However, no other treatment-related clinical signs were observed. In addition, no treatment-related changes were observed in motor activity or in the functional observational battery.

No treatment-related effects were observed in any of the hematological parameters investigated following 4 weeks of treatment with Hydrotreated C5s. However, when blood chemistry parameters were evaluated, a statistically significant lower group mean triglyceride level was observed in female rats receiving 3,033 and 8,502 ppm Hydrotreated C5s. A similar effect was not observed in male rats. As the triglyceride levels of the female controls were slightly greater than historical control values, and as no other related changes were apparent, this change is likely to be of no clinical significance.

Organ weight measurements revealed that bodyweight-adjusted group mean kidney weights were increased in all treated groups when compared with controls, with statistical significance being attained for males receiving 3,033 or 8,502 ppm Hydrotreated C5s. Bodyweight-adjusted mean kidney weights were increased in all groups of treated females.

No treatment related macroscopic changes were noted in any of the exposed animals. However, some pathological changes were noted upon microscopic observation. All male rats exposed to Hydrotreated C5s displayed pathological effects in the kidney as evidenced by renal cortical tubules containing hyaline droplets. Severity increased with increasing exposure concentration. This finding correlates with the increased bodyweight adjusted kidney weights reported in all of the male exposure groups. Basophilic cortical tubules were also increased in incidence and severity in all male exposure groups, and in the female 3,033 and 8,502 ppm groups compared to the air control groups.

In the liver, minimal centrilobular hepatocyte hypertrophy was found at a statistically significant incidence in the high dose male group, i.e., 8,502 ppm. This finding also correlates with the increased bodyweight adjusted liver weights reported in the male 8,502 ppm exposure group. Centrilobular hepatocyte hypertrophy along with slightly elevated liver weights are considered to be adaptive rather than adverse effects. The single cases in the male 992 ppm group and the female 3,033 ppm exposure group are not considered to be toxicologically significant.

In the nasal turbinates, atrophy/disorganization of the olfactory epithelium was found in several animals in the male high dose exposure group, i.e., 8,502 ppm, and in the female mid- and high-dose exposure groups, i.e., 3,033 and 8,502 ppm. Although the incidences had not achieved statistical significance it is considered that they are above background levels, and the finding is considered to be treatment-related in these groups.

In summary, a No Observed Effect level (NOEL) was not achieved in males; in females the NOAEL is 992 ppm. The repeated dose toxicity data are summarized in Table 13.

Conclusion

As part of the HPV Program, repeated dose studies have been conducted on the major components and two complex streams from the C5 Non-cyclics Category, including isoprene, 2-methyl-2-butene, Pyrolysis C5s stream and Hydrotreated C5s stream. The repeated dose studies conducted with isoprene demonstrate clear species differences between rats and mice in susceptibility to isoprene. For example, in rats, there were no observable toxicological effects at any dose following the 2-week repeated dose exposure. However, in mice, exposure to isoprene for 2 weeks induced changes in hematological parameters, body and organ weights and produced microscopic lesions in certain tissues at levels as low as 438 ppm. Similarly, in the 13-week study, no toxicological effects were evident in rats exposed up to 7,000 ppm isoprene for 13 weeks. However, in mice, hematological and histopathological changes were observed at exposures of 700 ppm and higher. This 13-week repeated dose inhalation study confirmed the species difference between rats and mice in susceptibility to isoprene. In the 26-week repeated dose study, there were no treatment related mortality, bodyweight change nor laboratory findings in rats. The only treatment related effect was an increased incidence and severity of interstitial cell hyperplasia of the testis at 7,000 ppm (highest dose) compared with controls. However, this lesion which was seen in all recovery groups also occurred at a high incidence in the controls and there was no concentration related trend. In mice exposed to isoprene for 26 weeks, there was reduced survival at the high dose from 18 weeks onwards. Mice exposed for 26 weeks and allowed to recover with no exposure for an additional 26 weeks showed evidence of spinal cord degeneration. After 26 weeks of exposure, spinal cord degeneration was evident only in mice exposed to 7,000 ppm. However, in recovery group animals after a further 26 weeks without exposure, spinal cord degeneration occurred with a statistically significant increased incidence above control in mice exposed to 70 ppm and above. In the chronic inhalation study (i.e., 80 weeks) conducted in mice, no clinical signs of toxicity were seen other than those associated with tumor development. Of note, in sharp contrast to what was observed in the 26-week study, there was no evidence of spinal cord degeneration in mice in the chronic 80-week study. This discrepancy was further evaluated in 2001 when the International Institute of Synthetic Rubber Producers' (IISRP) Isoprene Scientific Oversight Committee enlisted the services of Dr. Robert Garman, a veterinary pathologist. Dr. Garman conducted an independent neuropathology review of the slides from the 26-week repeat dose study in mice. This review confirmed the presence of minimal to mild degenerative changes within the sections of spinal cord from mice in the highest dose group, i.e., 7,000 ppm that had been necropsied immediately after the six month exposure period. However, no significant differences were found between the appearance of the spinal cord sections from the mice in the 0 ppm; 70 ppm; 220 ppm; and 7,000 ppm groups that were necropsied immediately after the six month recovery period during which time there had been no additional exposures to isoprene. This review supports the likelihood of recovery of the mice from the mild degenerative lesions found immediately after the six month isoprene exposure. Confirmation of this finding by the NTP is still pending.

2-Methyl-2-butene was tested in a 28-day repeat dose study in rats. Although some general effects were observed in this study, these effects were slight and were most apparent in those animals exposed to the highest dose, i.e., 7,000 ppm, and to a lesser extent to those exposed to 2,000 ppm.

The Pyrolysis C5 stream was also tested in a 28-day repeat dose study in rats at concentrations up to 1,000 ppm. No general systemic effects were observed during the routine clinical examination or during the functional observational battery. Slight histopathological changes were observed in the livers of the high dose rats. In the male rats, however, a higher kidney weight and incidence of cortical tubules with hyaline droplets was apparent in all treatment groups. High dose males also showed associated kidney lesions. No pathological changes were apparent in the kidneys of treated females. The kidney effects observed in male rats is a well studied phenomenon known as "light hydrocarbon nephropathy." This phenomenon has been extensively evaluated by the US

Environmental Protection Agency (EPA). The EPA has determined that "light hydrocarbon nephropathy" is a male rat-specific phenomenon and has no relevance for human risk assessment.

The Hydrotreated C5 stream was tested in a 28-day repeat dose study in rats at concentrations up to 8,502 ppm. Other than lethargy and increased salivation observed in the high dose animals, i.e., 8,502 ppm, no other treatment-related clinical signs were noted. In addition, no treatment-related changes were observed in motor activity or in the functional observational battery. No macroscopic changes were noted in any of the animals. However, male rats in all treatment groups again showed evidence of "light hydrocarbon nephropathy." Other effects included some minimal changes in the livers of high dose male rats and some atrophy/disorganization of the olfactory epithelium in the nasal turbinates in both high dose males and females as well as mid-dose females.

Table 13. Summary of Repeated Dose Toxicity Data for the C5 Non-cyclics Category

CAS RN and Substance Name	Test Organism	Exposure Duration (weeks)	Exposure-Related Effects	NOAEL (ppm)
78-79-5 Isoprene	Rat	2	None	7,000
78-79-5 Isoprene	Mouse	2	Changes in hematological parameters, body & organ weights, & observable microscopic lesions in certain tissues	438
78-79-5 Isoprene	Rat	13	None	7,000
78-79-5 Isoprene	Mouse	13	Hematological & histopathological changes	220
78-79-5 Isoprene	Rat	26	Interstitial cell hyperplasia of the testis	2,200
78-79-5 Isoprene	Mouse	26	Spinal cord degeneration increased at 70 ppm & above	<70
513-35-9 2-Methyl-2-Butene	Rat	4	Slight increase in severity of myocardial inflammatory heart lesions & in cortical/medullary tubular basophilia in the kidneys	580
68476-55-1 Pyrolysis C5	Rat (female)	4	Centrilobular hepatocyte hypertrophy along with slightly elevated liver weights are considered to be adaptive rather than adverse effects	1,000
68476-55-1 Pyrolysis C5	Rat (male)	4	Centrilobular hepatocyte hypertrophy along with slightly elevated liver weights are considered to be adaptive rather than adverse effects; effects consistent with light hydrocarbon nephropathy were also observed*	1,000
68602-79-9 Hydrotreated C5	Rat (female)	4	Atrophy/disorganization of the olfactory epithelium in the nasal turbinates; basophilic cortical tubules	992
68602-79-9 Hydrotreated C5	Rat (male)	4	Basophilic cortical tubules; Hyaline droplet nephropathy; effects consistent with light hydrocarbon nephropathy were also observed*	<992

* The EPA has determined that "light hydrocarbon nephropathy" is a male rat-specific phenomenon and has no relevance for human risk assessment.

4.1.4 Mutagenicity

In vitro Studies

Isoprene

Isoprene was tested in an Ames assay in four strains of *Salmonella typhimurium* (i.e., TA98, TA100, TA1535, TA1537) with and without metabolic activation (Mortelmans *et al.*, 1986). The preincubation modification of the Salmonella assay was used to test isoprene in these four different strains of *Salmonella* in the presence and absence of Aroclor 1254-induced rat and hamster liver S-9. Five dose levels plus control were tested (i.e., 0; 100; 333; 1,000; 3,333; and 10,000 µg/plate) with three plates per dose level. The high dose (10,000 µg/plate) was limited by toxicity. Concurrent positive controls were also tested with and without metabolic activation. The assay was repeated less than one week after completion of the initial test.

In this study, isoprene was not mutagenic in any of the four strains of *Salmonella* tested either in the presence or absence of Aroclor-induced rat or hamster liver S9.

The two isoprene monoepoxides, 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene and the isoprene diepoxide, 2-methyl-1,2,3,4-diepoxbutane were tested in two strains of *Salmonella typhimurium* (i.e., TA98 and TA100) without metabolic activation (Gervasi and Longo, 1990). These epoxides were assayed with the standard-plate incorporation test, without metabolic activation since they are direct-acting compounds. The two monoepoxide metabolites were not mutagenic. However, the diepoxide metabolite, 2-methyl-1,2,3,4-diepoxbutane was mutagenic in strain TA100 with a linear dose-effect relationship.

Concentrations of 0.25 to 25% isoprene monomer were subjected to Ames mutagenicity testing using vapor phase exposures (Huntington Life Sciences, Ltd., 2003a). To investigate possible species differences in metabolism, liver enzymatic preparations (S-9 and microsomes) were employed from uninduced B6C3F1 male mice. A positive control, vinyl chloride, provided evidence of both the mutagenic capabilities of the bacteria as well as activity of the liver enzymes. *Salmonella* strains TA1535, TA1537, TA98, and TA100 were employed as were *E. coli* WP2 uvrA (pKM101) bacteria. Criteria for a positive response included (a) evidence of dose-responsiveness, and (b) an increase in revertants of treated plates versus controls at two times for all strains except TA1535 & TA1537, which required a 3-fold increase. The maximal increase of revertant rates observed in isoprene-exposed bacteria was 1.7 for the TA1535 strain, and 1.6 in *E. coli*, both in the presence of S-9 activation. Vinyl chloride induced increases in revertant rates in these assays of 29- and 5.7-fold relative to negative controls, respectively. These results for isoprene indicate negligible mutagenic activity towards bacteria under the stated test conditions.

In conclusion, the two monoepoxide metabolites of isoprene are non-mutagenic, while the diepoxide metabolite is active. The potential for genotoxic effects of isoprene in rodents and other species is reasonably concluded to be based upon the chemical's metabolic profile in a species-dependent manner.

Isoprene was tested in an *in vitro* Sister Chromatid Exchange (SCE) assay in mammalian cells (Galloway *et al.*, 1987). In this study, isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of SCEs both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent, positive controls, and four doses of isoprene. The doses tested were 50; 160; 500; and 1,600 µg/ml (without S9) and 160; 500; 1,600; and 5,000 µg/ml (with S9). A single flask per dose was used. All slides were scored blind and those from a single test were read by the same person. Fifty 2nd division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Isoprene was not genotoxic in this study as no increases in SCEs were noted in the cultured CHO cells treated with isoprene, with or without S9.

Isoprene was also tested in an *in vitro* Mammalian Chromosomal Aberration Test (Galloway *et al.*, 1987). In this study, isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent, positive controls, and three doses of isoprene. The doses tested were 1,600; 3,000; and 5,000 µg/ml. A single flask per dose was used. All slides were scored blind and those from a single test were read by the same person. Two hundred 1st-division metaphase cells were scored for chromosomal aberrations at each dose level.

Isoprene was not genotoxic in this study as no increases in chromosomal aberrations were noted in cultured CHO cells treated with isoprene, with or without S9.

2-Methyl-2-Butene

2M2B was tested in an Ames assay in 5 strains of *Salmonella typhimurium* (i.e., TA1535, TA 1537, TA1538, TA 100 and TA98), and in 2 strains of *Escherichia coli* (i.e., WP₂ and WP₂uvrA) in the presence and absence of rat liver S-9 (Dean *et al.*, 1985). Five dose levels were tested, with three plates per dose level. Concurrent positive and solvent controls were also tested with and without metabolic activation (rat liver S9). Two replicate assays were performed on different days to confirm the reproducibility of the results. 2M2B was not mutagenic in any of the five strains of *Salmonella* or in the two strains of *E. coli* tested in the presence or absence of metabolic activation

2M2B was tested in a *Saccharomyces cerevisiae* gene conversion assay (Dean *et al.*, 1985). In this assay liquid suspension cultures of *S. cerevisiae* in dilute growth medium were dosed with 0.2, 2, 10, 20, or 50 mg/ml of 2M2B in ethanol to give a final concentration of 0.01; 0.1; 0.5; 1.0; or 5.0 mg/ml. After 18 hours of incubation with shaking at 30°C either in the presence or absence of rat liver S9 fraction, the cultures were seeded onto the appropriate culture media for the selection of revertant colonies. After 3 days incubation at 30°C, the numbers of revertant colonies were counted. In this assay, a test material is considered to be mutagenic if the number of revertants per 10⁶ survivor cells in the treated plates is greater than twice the control value. This did not occur with 2M2B at any of the concentrations tested either with or without metabolic activation. Thus, 2M2B was not mutagenic to yeast cells under the conditions of this assay.

2M2B was tested in a rat liver cytogenetics assay (Dean *et al.*, 1985). In this assay cultures of rat liver cells (RL₄) were prepared in glass prescription bottles (200 ml) at an initial cell density of 10⁶ cells using 25 ml of culture medium, (Minimum Essential medium + 10% fetal calf serum + 1% non-essential amino acids). The cultures were incubated at 30°C for 24 hours to allow active growth to commence; freshly prepared solutions of 2M2B were then added at concentrations of 12.5; 25; or 50 µl/ml. These concentrations were selected on the basis of a previously conducted cytotoxicity test which determined the concentration producing 50% growth inhibition (i.e., 100 µl/ml), and appropriate dilutions of this concentration (i.e., 0.125; 0.25; and 0.5%) were used. Positive control cultures using 1 µg/ml 7,12-dimethyl benzantracene were run in parallel. The chromosome preparations were randomly coded and 100 cells from each culture were analysed microscopically for chromosome changes. Based on the results of the metaphase chromosome analysis, 2M2B did not induce chromosome damage in cultured rat liver cells (RL₄) exposed for 24 hours to concentrations of 12.5; 25.0; and 50 µl/ml, respectively. Thus, 2M2B was not genotoxic under the conditions of this assay.

Pyrolysis C5

The mutagenic potential of Pyrolysis C5 was assessed in 4 strains of *Salmonella typhimurium* (i.e., TA 1535, TA 1537, TA 98, and TA 100) and in a tryptophan dependent mutant of *Escheria coli*, strain WP2uvrA/pKM101 (Huntingdon Life Sciences Ltd, 2002c). The bacteria were exposed to Pyrolysis C5 in vapor phase. These studies were conducted in accordance with OECD Test Guideline 471, EC Commission Directive 2000/32/EC Annex 4D-B. 13/14 and US EPA OPPTS 770.5100.

Both studies were performed in the presence and absence of liver preparations from Aroclor 1254-induced rats (S9 mix). Concentrations of Pyrolysis C5 up to 0.525% v/v (5250 ppm; 50% of the Lower Explosive Limit) were tested in the mutation tests in vapor phase. Agar plates, seeded with the tester strains, were exposed to the test substance for 48 hours at 37 degrees C, then incubated in the absence of the test substance for a further 24 hours. Revertant colony numbers were counted after incubation. No signs of toxicity were observed towards the tester strains in either mutation test. All bacterial lawns were normal.

No evidence of mutagenic activity was seen at any concentration of Pyrolysis C5 in either mutation test. The responses of the positive controls were all within historical data ranges, with the exception of dichloromethane where responses exceeded the historical data range. Positive control chemicals used in this study that required metabolic activation were benzo(a)pyrene and 2-aminoanthracene. Direct-acting positive controls included 2-nitrofluorene, sodium azide, 9-aminoacridine, and dichloromethane.

In conclusion, under the test conditions employed, Pyrolysis C5 showed no evidence of mutagenic activity in either bacterial system.

Hydrotreated C5s

The mutagenic potential of Hydrotreated C5s was assessed in 4 strains of *Salmonella typhimurium* (i.e., TA 1535, TA1537, TA98, and TA100) and in a tryptophan dependent mutant of *Escherichia coli*, strain WP2uvrA/pKM101 (Huntingdon Life Sciences Ltd, 2003h). The bacteria were exposed to Hydrotreated C5s in vapor phase. These studies were conducted in accordance with OECD Test Guideline 471, EC Commission Directive 2000/32/EC Annex 4D-B. 13/14 and US EPA OPPTS 770.5100.

Both studies were performed in the presence and absence of liver preparations from Aroclor 1254-induced rats (S9 mix). Concentrations of Hydrotreated C5s up to 0.85% v/v (50% of the Lower Explosive Limit) were tested in the mutation test. The lower concentrations used were separated by ca half- \log_{10} intervals. No signs of toxicity were observed towards the tester strains in either mutation test.

No evidence of mutagenic activity was seen at any concentration of Hydrotreated C5s in either mutation test. The concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations. Positive control chemicals used in this study were benzo(a)pyrene, 2-aminoanthracene, sodium azide, 2-nitrofluorene, 9-aminoacridine, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide and dichloromethane.

In conclusion, under the test conditions employed, Hydrotreated C5s showed no evidence of mutagenic activity in this bacterial system.

*In vivo Studies***Isoprene**

An *in vivo* Sister Chromatid Exchange (SCE) study was conducted in B6C3F1 mice by Tice *et al.* (1988). In this study, 15 male B6C3F1 mice per group were exposed for 12 days, 6 hours/day to 0; 438; 1,750; or 7,000 ppm of isoprene by inhalation. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours prior to sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of SCE, 5 mice per exposure group were euthanized 24 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Twenty-five second-division metaphase cells were scored for SCEs from 4 mice/group.

Exposure to isoprene for 6 hours/day at 0; 438; 1,750; or 7,000 ppm for 12 days induced a significant increase in the frequency of SCEs in bone marrow cells at all three dose levels (4.40 at 0 ppm; 14.84 at 438 ppm; 11.61 at 1,750 ppm; and 13.98 at 7,000 ppm). The increased SCE responses in the exposed groups were not statistically different from each other. There were no significant clinical signs or mortality throughout the study.

In this study, isoprene was found to be genotoxic and cytotoxic to mouse bone marrow *in vivo*, inducing SCE, inhibiting cellular proliferation, and suppressing the rate of erythropoiesis. The lack of significant difference in SCEs among the three exposed groups suggests a saturation of the metabolic capacity of male mice to form reactive species.

A Mammalian Bone Marrow Chromosomal Aberration test was conducted in B6C3F1 mice by Tice *et al.* (1988). In this study, 15 male B6C3F1 mice per group were exposed for 12 days, 6 hours/day to 0; 438; 1,750; or 7,000 ppm isoprene by inhalation. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours before sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of chromosomal aberrations, 10 mice per exposure group were killed 17 to 20 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Fifty first-division metaphase cells were scored for aberrations from 8 mice/group. Additionally, 100 randomly selected metaphase cells per slide were scored for replication history to provide data on cell generation time, a measure of cell proliferation kinetics. The percentage of cells in metaphase among 1,000 cells/sample was used to calculate the mitotic index.

In this study, exposure of mice to isoprene for 6 hours/day at concentrations of 0; 438; 1,750; or 7,000 ppm for 12 days did not induce a statistically significant increase in the frequency of chromosomal aberrations in bone marrow cells. The incidence of bone marrow cells with chromosomal aberrations was slightly elevated in the exposed groups compared to the control but these increases were not statistically significant. Mitotic index data indicated no significant change in the percentage of bone marrow cells engaged in division, although the 7,000 ppm group was slightly increased compared to the controls. Analysis of the average generation time showed a statistically significant lengthening of the cell cycle duration of proliferating cells in the 7,000 ppm group.

In conclusion, although the incidence of bone marrow cells with chromosomal aberrations in male mice treated with isoprene for 12 days were slightly elevated at all dose groups compared to controls, none of the increases were statistically significant.

A Mammalian Erythrocyte Micronucleus Test was conducted in B6C3F1 mice by Tice *et al.* (1988). In this study, 15 male B6C3F1 mice per group were exposed by inhalation to isoprene at concentrations of 0; 438; 1,750; and 7,000 ppm, 6 hours/day for 12 days. Approximately 24 hours following the last exposure, peripheral blood samples were obtained from each animal by tail snip, air-dried immediately and fixed with methanol. One thousand polychromatic erythrocytes (PCEs) and 1,000 normochromatic erythrocytes (NCEs) were scored per animal for frequency of micronucleated erythrocytes (MN). The percentage of PCEs in 1,000 erythrocytes was also determined as a measure of isoprene-induced toxicity.

In this study, exposure to isoprene induced a statistically significant increase in the frequency of MN-PCEs and NCEs in male mice at all exposure concentrations tested. There was also a dose-related decrease in the percentage of PCEs, a measure of the rate of erythropoiesis. There were no significant clinical signs or mortality throughout the study.

In conclusion, isoprene was genotoxic to mouse bone marrow *in vivo*. A decrease in the percentage of PCEs was also observed which suggests that erythropoiesis was also being suppressed.

A rat lung fibroblast Micronucleus Test was conducted in Fischer 344 rats by the National Toxicology Program (1997). Groups of 10 male and 10 female rats per group were exposed for 4 weeks to 0; 220; 700; or 7,000 ppm isoprene by inhalation for a total of 17 to 19 exposures. The rats received at least two consecutive days of exposure prior to sacrifice and lung cell isolation. Lung fibroblasts were isolated and cultured in single-chamber slides for 72 hours. The slides were fixed and stained with acridine orange and 1,000 binucleated cells on each of two slides per animal were scored. The number of mononucleated cells and micronuclei were recorded following a standard scoring criteria.

There were no statistically significant differences between the male or female exposed and control groups for micronucleated rat lung fibroblasts. There were no significant clinical signs or mortality during the exposure period.

In conclusion, isoprene was not genotoxic in this study. No significant increase in the frequency of micronucleated lung fibroblasts was observed in male and female rats exposed to isoprene for 4 weeks.

2-Methyl-2-Butene

2M2B was evaluated for its ability to induce micronuclei in bone marrow polychromatic erythrocytes (PCEs) in male B₆C₃F₁ mice (Exxon Biomedical Sciences, 1991a). Male mice (10/group) were exposed 6 hours a day for 2 consecutive days to 0; 1,005; 3,207; or 9,956 ppm 2M2B by inhalation. Another group of 10 male mice was exposed to 1,000 ppm 1,3-butadiene and served as the positive control. The mean micronucleated PCE values were 4.2; 16.6 and 36.1 at 1,005; 3,207; and 9,956 ppm, compared to 3.4 micronucleated PCEs for the negative control. 2M2B induced statistically significant ($p < 0.01$) and dose related increases in micronucleated PCEs at 3207 and 9956 ppm. A statistically significant ($p < 0.01$) decrease in the %PCEs, which is a measure of hematotoxicity, was only observed at 9956 ppm. The positive control produced a statistically significant increase in micronucleated PCEs (29.7) and decrease in %PCEs (44.5%). Under the conditions of this study, exposure to 2M2B > 3207 ppm induced statistically significant increases in micronucleated polychromatic erythrocytes in male mice; the No Observed Effect Level was 1,005 ppm.

2M2B was evaluated for its ability to induce micronuclei in bone marrow polychromatic erythrocytes (PCEs) in male CrlCDBR rats (Exxon Biomedical Sciences, 1991b). Male rats (10/group) were exposed 6 hours a day for 2 consecutive days to 0; 1,005; 3,207; or 9,956 ppm 2M2B by inhalation. 2M2B induced statistically significant ($p < 0.01$) and dose related increases in

micronucleated PCEs at 3,207 and 9,956 ppm. The mean micronucleated PCE values were 2.2; 4.2; and 4.9 at 1,005; 3,207; and 9,956 ppm, respectively, compared to 2.7 for the negative control (air). The mean %PCEs at 1,005; 3,207; and 9,956 ppm (48.6; 51.0; and 49.8%, respectively) were slightly decreased from the negative control (54.9%), but they were not different from each other and did not show evidence of dose-response. Therefore, the biological significance of this observation is unclear. Under the conditions of this study, inhalation exposure to 2M2B \geq 3,207 ppm induced small but statistically significant increases in micronucleated polychromatic erythrocytes in male rats; the No Observed Effect Level was 1,005 ppm.

Pyrolysis C5

Pyrolysis C5 was evaluated for its ability to induce micronuclei in bone marrow cells of CD-1 male mice (Huntingdon Life Sciences Ltd., 2002d). This study was conducted in accordance with OECD Test Guideline 474, US EPA OPPTS 870.5395 and EC Directive 2000/32/EC, L 136/50.

In this study, groups of seven male CD-1 male mice were exposed to Pyrolysis C5 for two 6-hour exposure periods approximately 24 hours apart at target exposure levels of 40, 150, and 500 ppm. The test substance and negative control (i.e., clean air) were administered by whole body inhalation exposure. The positive control group was dosed by oral gavage with mitomycin C at 12 mg/kg bodyweight. All animals were sacrificed approximately 24 hours following the second exposure period (24 hours after the oral dose for the positive control group).

Bone marrow smears were prepared from all animals and examined to evaluate the incidence of micronuclei in 2,000 polychromatic erythrocytes (PCE) per animal. The proportion of PCE was assessed by examination of at least 1,000 erythrocytes.

No statistically significant increase in the incidence of micronucleated PCE were observed in the Pyrolysis C5s exposed animals compared with the negative control values. The positive control treatment induced a significant increase.

In conclusion, Pyrolysis C5 did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered by whole body inhalation to CD-1 male mice in this study.

Hydrotreated C5s

Hydrotreated C5s was evaluated for its ability to induce micronuclei in bone marrow cells of CD-1 male mice (Huntingdon Life Sciences Ltd., 2003i). This study was conducted in accordance with OECD Test Guideline 474, EC Commission Directive 2000/32/EC Annex 4C -B.12 and US EPA Health Effects Test Guidelines - EPA 712-C-98-226.

In this study, the mice were exposed to the test material for two 6-hour exposure periods approximately 24 hours apart at target exposure levels of 2,000; 4,000; and 8,000 ppm. The test substance and negative control (i.e., clean air) were administered by whole body inhalation exposure. The positive control group was dosed orally with mitomycin C at 12 mg/kg bodyweight on one occasion, approximately 24 hours before termination.

Bone marrow smears were obtained from seven male animals in the negative control group, each of the test substance groups and five male animals in the positive control group 24 hours after the final dose. One smear from each animal was examined for the presence of micronuclei in 2,000 immature erythrocytes. The proportion of immature erythrocytes was assessed by examination of at least 1,000 erythrocytes from each animal. A record of the incidence of micronucleated mature erythrocytes was also kept.

The target vapor concentrations of Hydrotreated C5s were 2,000; 4,000; and 8,000 ppm for the low, intermediate, and high dose groups, respectively. Analysed mean chamber concentrations for the

two exposure periods were 1,927; 3,729; and 7,894 ppm, indicating that the test atmospheres were within the acceptable ranges of 4, 7, and 1%, respectively, from target.

No statistically significant increases in the frequency of micronucleated immature erythrocytes were observed in mice following two 6-hour whole body inhalation exposures of Hydrotreated C5s at 2,000; 4,000; and 8,000 ppm, compared to negative control values ($P > 0.01$ in each case).

A small statistically significant decrease in the proportion of immature erythrocytes was observed in animals exposed to Hydrotreated C5s. The proportion of immature erythrocytes was within the normal range of variability for this species and was not considered to be of any biological importance.

In conclusion, Hydrotreated C5s did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered by whole body inhalation to CD-1 male mice in this study.

Conclusion

Isoprene was genotoxic to mouse bone marrow *in vivo*. Exposure of B6C3F1 mice to isoprene resulted in a statistically significant increase in sister chromatid exchanges and bone marrow micronuclei. However, isoprene did not produce an increase in micronucleated lung fibroblasts in exposed F344 rats. Isoprene was not genotoxic in any of the *in vitro* assays conducted. Two monoepoxide metabolites were not mutagenic in any of the *Salmonella* strains tested. The diepoxide metabolite, 2-methyl-1,2,3,4-diepoxybutane was mutagenic in *Salmonella* strain TA100. Isoprene did not produce statistically significant increases in either sister chromatid exchanges or in chromosomal aberrations in exposed Chinese Hamster Ovary Cells.

2M2B is not mutagenic *in vitro*. It did not induce gene mutations in reverse mutation assays conducted in *Salmonella typhimurium* and *Escherichia coli* either in the presence or absence of metabolic activation. 2M2B did not produce revertant colonies in a gene conversion assay conducted in *Saccharomyces cerevisiae* and it did not induce chromosome damage in cultured rat liver cells.

2M2B was mutagenic at high exposure concentrations (at exposures greater than 3,207 ppm (9,199 mg/m³)) when tested *in vivo* for its ability to induce micronuclei in bone marrow polychromatic erythrocytes (PCEs) in both mice and rats.

The Hydrotreated C5 stream was not mutagenic either *in vitro* or *in vivo*. It did not induce gene mutations in reverse mutation assays conducted in *Salmonella typhimurium* and *Escherichia coli* either in the presence or absence of metabolic activation. In addition, it did not show any evidence of causing chromosome damage or bone marrow toxicity when administered by whole body inhalation to CD-1 male mice in an *in vivo* micronucleus assay.

4.1.5 Carcinogenicity

Studies in Animals

Isoprene

Inhalation

A 26-week inhalation exposure study was conducted with isoprene in F344 rats and B6C3F1 mice (Melnick et al., 1994). In this study, groups of 40 male B6C3F1 mice and Fischer 344 rats were exposed to 0; 70; 220; 700; 2,200; or 7,000 ppm isoprene vapor by inhalation for 6 hours/day, 5 days/week for 6 months. At the end of the 26-week exposure period, 10 rats and 10 mice/group were sacrificed and evaluated. The remaining animals were allowed to recover for an additional 26

weeks without exposure at which time they were also sacrificed and evaluated. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically.

Interstitial cell hyperplasia of the testis was observed in male rats after 26 weeks of exposure to 7,000 ppm isoprene; following the 26-week recovery period, the only effect in rats was a marginal increase in benign testicular interstitial cell tumors in the 7,000 ppm group. The survival of mice was reduced in the 7,000 ppm group; early deaths were attributed to various neoplastic lesions and moribund sacrifices due to hindlimb paralysis. In male mice, incidences of malignant neoplastic lesions in the liver, lung, forestomach, and Harderian gland were significantly increased following the 26-week exposure and 26-week recovery periods at 700 ppm and higher exposures

Thus, in this repeated dose study, isoprene was carcinogenic to the liver, lung, forestomach and Harderian gland of male mice after 26 weeks exposure and 26 weeks recovery. In contrast, the only effect observed in male rats was a marginally increased incidence of benign testicular adenomas at the highest exposure level (7,000 ppm).

In a chronic oncogenicity study, B6C3F₁ mice were exposed to isoprene by inhalation for either 4 or 8 hours/day, 5 days/week for 20, 40 or 80 weeks (Placke *et al.*, 1996). Twelve groups of 50 male B6C3F₁ mice were exposed to 0; 10; 70; 140; 280; 700; or 2,200 ppm of isoprene vapor for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period, leading to a total planned study length of 104 weeks. Female mice (50/group) were exposed to 0, 10 and 70 ppm of isoprene, 8 hours/day for 80 weeks and also held for observation through week 104. Selected groups of mice were removed at the end of 20 or 40 weeks of exposure, and were held in holding chambers for the duration of the 80-week exposure period. At the end of 80 weeks, all surviving animals were moved to a holding room through study week 104 and then necropsied beginning in study week 105.

Isoprene produced exposure-related increases in the incidence of liver, lung, Harderian gland and forestomach tumors, as well as increased incidences of hemangiosarcomas and histiocytic sarcomas. These results were similar to the profile of tumors seen in 1,3-butadiene except for the absence of the early onset of T-cell lymphoma seen with butadiene (NTP, 1993). In this study, 10 ppm was a No Observed Adverse Effect Level (NOAEL) for the carcinogenic effects. The Lowest Observed Adverse Effect Level (LOAEL) for tumors in this study was between 70 and 140 ppm. Biostatistical analysis of the tumor incidence data indicated that cumulative exposure was not an adequate predictor of risk. The same cumulative exposure could be more or less damaging, depending upon how it was administered over time. With respect to nonneoplastic lesions, there were no apparent effects on motor function and no exposure-related lesions in the spinal cord at any concentration. This is in sharp contrast to what was observed in the NTP subchronic study where partial hindlimb paralysis and spinal cord degeneration was observed in mice exposed to 70 ppm for 6 months.

A chronic inhalation oncogenicity study was also conducted in rats. In this study, groups of 50 male and female F344/N rats were exposed to 220; 700; or 7,000 ppm isoprene by inhalation, 6 hours per day, 5 days per week, for 104 weeks (NTP, 1999). The survival of exposed males and females was similar to that of the chamber controls. Mean body weights of exposed male and female rats were similar to those of the chamber controls throughout the study.

Exposure-related increases in the incidences of mammary gland fibroadenoma occurred in male rats in all exposure groups. Mammary gland fibroadenoma is considered to be a very rare tumor in male rats. The incidences of fibroadenoma in 7,000 ppm males and all groups of exposed females were significantly greater than those in the chamber control groups. The incidences of fibroadenoma in all exposed groups of males and females, of multiple fibroadenoma in 7,000 ppm males, and in all groups of exposed females exceeded the historical control ranges. The incidences

of renal tubule adenoma in 700 and 7,000 ppm males, and the incidence of renal tubule hyperplasia in 7,000 ppm males were significantly greater than those in the chamber controls. In addition, an exposure-related increase in the incidences of interstitial cell adenoma of the testis occurred in male rats. The incidences of bilateral interstitial cell adenoma, and of unilateral and bilateral interstitial cell adenoma (combined) of the testis in 700 and 7,000 ppm males were significantly greater than those in the chamber controls. The incidences of interstitial cell adenoma in 700 and 7,000 ppm males exceeded the historical control range. Several rare neoplasms including benign astrocytoma, malignant glioma, and malignant medulloblastoma, granular cell tumor and meningeal sarcoma were observed in the brain of exposed female rats. The neoplasms rarely occur in historical chamber controls. However, the fact that they are of different cell types makes it difficult to determine if they are truly exposure-related.

In summary, isoprene exposures were associated with increases in rates of benign tumors in the testes and kidney (male), and mammary gland (male and female). No significant increases were seen for malignant tumors in this study. For this reason, and the fact that brain tumors in females were of several distinct cell types, the overall level of evidence presented for the carcinogenicity of isoprene in rats is, at most, limited. In spite of this, the NTP concluded that under the conditions of this 2-year inhalation study, there was *clear evidence of carcinogenic activity* of isoprene in male F344/N rats based on increased incidences of mammary gland neoplasms, renal tubule adenoma, and testicular adenoma. They also concluded that there was *some evidence of carcinogenic activity* of isoprene in female F344/N rats based on increased incidences and multiplicity of mammary gland fibroadenoma. A low incidence of rare brain neoplasms in exposed female rats may have been due to exposure to isoprene. In summary, based on the results of the carcinogenicity studies conducted in mice and rats, the NTP listed isoprene as "reasonably anticipated to be a human carcinogen" in the 9th Report on Carcinogens.

Conclusion

There is clear evidence of carcinogenicity of isoprene in mice due to the induction of malignant tumors in two separate studies. Isoprene produced exposure-related increases in the incidence of malignant neoplasms in the liver, lung, Harderian gland and forestomach of mice, as well as increases in the number of hemangiosarcomas and histiocytic sarcomas. In rats, on the other hand, there were no significant increases in the incidence of malignant tumors. In rats isoprene exposures were associated with increases in the rates of benign tumors in the testes and kidney (male) and mammary gland (male and female). Although single incidences of several rare brain neoplasms were observed in female rats, the fact that they were of several distinct cell types, makes it difficult to determine if they are truly exposure related.

4.1.6 Toxicity for Reproduction

Studies in Animals - Reproductive and Developmental Toxicity (Tables 14 and 15)

Isoprene

Inhalation

No guideline reproductive studies have been conducted with isoprene. Histopathology of the reproductive organs was evaluated in repeated dose inhalation studies conducted in F344 rats and B6C3F1 mice at exposure concentrations of 0; 70; 220; 700; 2,200; and 7,000 ppm for 6 hours/day, 5 days/week for 13 weeks and 26 weeks (Melnick *et al.*, 1994). In addition, sperm motility and vaginal cytology evaluations were performed on all rats and mice exposed to 0; 70; 700; or 7,000 ppm in the 13-week study.

No treatment-related effects were observed in rats exposed to isoprene for 13 weeks. Following 26 weeks of exposure, the only effect in rats was an increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7,000 ppm group. Following the 26 week recovery period, the incidence of interstitial cell adenoma was slightly greater in male rats exposed to 700 ppm or higher. Based on the findings of increased hyperplastic lesions at 26 weeks and adenomas following the 26-week recovery, the early development of interstitial cell-proliferative lesions was considered to be exposure-related.

In mice exposed to isoprene for 13 weeks, testicular weight was reduced 35% in the 7,000 ppm group, and minimal morphological changes (seminiferous tubular atrophy) were detected in 2/10 mice. Compared to controls, male mice in the 700 and 7,000 ppm groups had 12% and 30% lower epididymal weights, 12% and 46% lower spermatid head counts, 12% and 46% lower sperm concentrations, and 6% and 23% reductions in sperm motility, respectively. The female mice exposed to 7,000 ppm exhibited estrous cycle lengths significantly longer than the control group (4.8 vs. 4.2 days). In summary, mice exposed to isoprene for 13 weeks exhibited significant effects at 700 ppm or higher, including increased estrous cycle length and testicular atrophy, and decreased epididymal weight, sperm head count, sperm concentration, and sperm motility.

Female Swiss CD-1 mice and Sprague-Dawley rats were exposed to 0; 280; 1,400; or 7,000 ppm isoprene for 6 hours/day, 7 days/week on gestational days 6 to 17 in mice or gestational days 6 to 19 in rats (NTP, 1989).

In rats, there was no adverse effect on the dam or offspring at any dose level, and there was no increase in malformations or variations. A slight, but not statistically significant, increase in the incidence of reduced vertebral ossifications (centra) was noted at 7,000 ppm. Thus, in rats 7,000 ppm was the NOAEL for both maternal and developmental toxicity.

In mice, 7,000 ppm isoprene significantly reduced maternal weight gain and uterine weight. Developmental toxicity was evident in mice as a statistically significant reduction in fetal bodyweight at the 280 ppm level for female fetuses and at the 1,400 ppm level for male fetuses. No embryotoxicity in the form of increased intrauterine death was present at any exposure level. Although there was no significant increase in the incidence of malformations, two fetuses with cleft palate were found, one in each of the two highest exposure groups (i.e., 1,400 and 7,000 ppm). Cleft palates were not detected in the control group. Increased incidences of variations (i.e., supernumerary ribs) were observed in the exposed groups. However, this skeletal variation is generally considered as a secondary effect of maternal toxicity or stress, and its significance is unclear. Thus, in mice, 1,400 ppm was the NOAEL for maternal toxicity. A NOAEL for developmental toxicity could not be determined in this study because effects (reduction in fetal bodyweight) were observed at the lowest exposure concentration tested (280 ppm).

2-Methyl-2-Butene

Inhalation

An OECD 422 combined repeated dose and reproduction/developmental toxicity study was conducted in Sprague Dawley rats (Huntingdon Life Sciences Ltd, 2003e). In this study a satellite group of 12 female rats was exposed to 0; 580; 2,000; or 7,000 ppm 2M2B by inhalation for approximately 6 hours/day for two weeks prior to breeding, during breeding and through day 19 of gestation. Males from the main study (discussed in section 3.1.5) were used to breed these females. The dams were allowed to deliver their litters, which were retained until lactation Day 4. During the study, clinical condition, bodyweight, food consumption, estrus cycles, mating performance, litter data, organ weights, and macroscopic pathology were undertaken.

Exposure of female rats for 2 weeks prior to mating and up to day 19 of gestation did not produce any evidence of reproductive or developmental toxicity. The estrus cycle was unaffected by exposure, and mating performance, fertility indices and gestation length were similar in all groups. There were no adverse effects upon survival or growth of the offspring *in utero* or up to day 4 of lactation. Thus, the No Observed Adverse Effect Level (NOAEL) for reproductive/developmental toxicity was 7,000 ppm.

Pyrolysis C5

Inhalation

An OECD 422 combined repeated dose and reproduction/developmental toxicity study was conducted in Sprague Dawley rats (Huntingdon Life Sciences Ltd, 2003f). In this study three groups of 12 female rats were exposed to 0; 100; 300; or 1,000 ppm Pyrolysis C5s by inhalation for approximately 6 hours/day for two weeks prior to mating, throughout mating and through to day 19 of gestation. The females were allowed to deliver their litters and rear their offspring to day 4 of lactation. During the study, clinical condition, bodyweight, food consumption, estrus cycles, mating performance, litter data, organ weights, and macroscopic pathology were undertaken.

Exposure of female rats for 2 weeks prior to mating and up to day 19 of gestation did not produce any evidence of reproductive or developmental toxicity. There were no treatment-related effects on estrus cycles or pre-coital interval. Mating performance and fertility were unaffected by treatment with all but one male and female pairing resulting in a viable pregnancy. The gestation length was similar in all groups and parturition was unaffected by treatment.

There were no effects on implantation counts or resultant litter size at birth and day 4. Post implantation loss and pup survival was unaffected by treatment. Pup bodyweight was not adversely affected. There were no effects on macroscopic examination of pups at day 4 of lactation.

In conclusion, the no effect level for reproductive/developmental toxicity in this screening study was 1,000 ppm.

Hydrotreated C5

Inhalation

An OECD 422 combined repeated dose and reproduction/developmental toxicity study was conducted in Sprague Dawley rats (Huntingdon Life Sciences, 2003g). In this study, three groups of twelve female rats were exposed by inhalation to target concentrations of 0; 1,000; 3,000; or 8,500 ppm Hydrotreated C5s for 6 hours/day 2 weeks prior to mating, throughout mating and through day 19 of gestation; their male partners were the same animals that were being concurrently treated for the repeated dose toxicity investigations. The females were allowed to deliver their litters and rear their offspring to day 4 of lactation. During the study, clinical signs, bodyweight, food consumption, estrus cycles, mating performance, litter data, organ weights and macroscopic pathology of the reproductive phase females were evaluated. The study mean analysed concentrations of Hydrotreated C5s over the duration of the study were 992; 3,033; or 8,502 ppm, respectively. These levels were in good agreement with the target exposure levels.

Clinical signs included salivation in high-dose females (i.e., 8,502 ppm) during a portion of the treatment period. No other treatment-related signs were noted during the study and there were no unscheduled deaths.

There were no treatment-related effects on the estrus cycles or on the pre-coital interval. Mating performance and fertility were unaffected by treatment with all but one male and female pairing

resulting in pregnancy and birth of a viable litter. The gestation length was similar in all groups and parturition was unaffected by treatment.

There were no adverse effects on implantation counts or resultant litter size at birth and day 4. Post implantation loss and pup survival was unaffected by treatment. Pup bodyweight was not adversely affected. There were no effects on macroscopic examination of pups at day 4 of lactation.

There were no treatment-related effects on organ weight and no macroscopic findings.

In conclusion, based on these results, the no effect level of Hydrotreated C5s for reproductive/developmental toxicity in this screening study was at least 8,502 ppm.

Conclusion

Isoprene did not produce any maternal or developmental toxicity in rats. However, both maternal and developmental toxicity were evident in mice. In mice, maternal weight gain and uterine weight were significantly reduced at the highest dose (i.e., 7,000 ppm). Significant reductions in fetal bodyweight were observed at the 280 ppm dose level for female fetuses and at the 1,400 ppm level for male fetuses. Thus, in this study, 1,400 ppm was the NOAEL for maternal toxicity. A NOAEL for developmental toxicity could not be determined as effects were observed at the lowest exposure concentration tested, i.e., 280 ppm. No significant effects were observed after histopathological evaluations of reproductive organs in rats except slight changes in the testis at the highest exposure level (7,000 ppm). However, significant effects were observed in mice exposed to isoprene concentrations of 700 ppm and higher, including increased estrous cycle length and testicular atrophy as well as decreased epididymal weight. In addition, decreased sperm head count, sperm concentration, and sperm motility were also observed.

OECD 422 combined repeated dose and reproduction/developmental toxicity studies were conducted on 2-Methyl-2-Butene, and the Pyrolysis C5 and Hydrotreated C5 streams. In all cases, exposure of rats to these materials did not produce any evidence of reproductive or developmental toxicity at any of the doses tested.

Table 14. Summary of Reproductive Toxicity Data for the C5 Non-cyclics Category

CAS RN and Substance Name	Test Organism	OECD Test Guideline	Exposure-Related Effects	NOAEL (ppm)
78-79-5 Isoprene	Rat	Melnick, 1994 (26 weeks)	Interstitial cell hyperplasia /adenoma of the testis	220
78-79-5 Isoprene	Mouse	Melnick, 1994 (13 weeks)	Decreased sperm head count, sperm concentration & sperm motility; increased estrous cycle length	70
513-35-9 2-Methyl-2-Butene	Rat	422	No exposure-related effects were observed	7,000
68476-55-1 Pyrolysis C5	Rat	422	No exposure-related effects were observed	1,000
68602-79-9 Hydrotreated C5	Rat	422	No exposure-related effects were observed	8,502

Table 15. Summary of Developmental Toxicity Data for the C5 Non-cyclics Category

CAS RN and Substance Name	Test Organism	OECD Test Guideline	Exposure-Related Effects	NOAEL (ppm)
78-79-5 Isoprene	Rat	NTP, 1989	No exposure-related effects were observed	7,000
78-79-5 Isoprene	Mouse	NTP, 1989	Significant reduction in fetal body weight at lowest dose tested	NE*
513-35-9 2-Methyl-2-Butene	Rat	422	No exposure-related effects observed	7,000
68476-55-1 Pyrolysis C5	Rat	422	No exposure related effects observed	1,000
68602-79-9 Hydrotreated C5	Rat	422	No treatment-related effects observed	8,502

* NE not established

4.2 Assessment Summary for Human Health

All members of the C5 Non-cyclics Category are expected to have a low order of acute toxicity. Isoprene and 2M2B have been shown to have a low order of acute toxicity in animals by both the oral and inhalation routes of exposure. In addition, 2M2B was shown to have a low order of acute toxicity by the dermal route of exposure.

Based upon read-across to 2M2B, all members of the C5 Non-cyclics Category would be expected to be mildly irritating to the skin and non-irritating to eyes. None of the materials in this category have been tested for respiratory tract sensory irritation potential .

Repeated dose studies have been conducted on two major components (isoprene and 2M2B) and two complex streams (Pyrolysis C5s and Hydrotreated C5s streams) from the C5 Non-cyclics Category. However, it is isoprene that has been studied most extensively. The repeated dose studies conducted by the National Toxicology Program (NTP) with isoprene demonstrate clear species differences between rats and mice in susceptibility to isoprene. For example, there were no observable toxicological effects at any dose following the 2-week repeated dose exposure to rats. In contrast, exposure of mice to isoprene for 2 weeks induced changes in hematological parameters, body and organ weights and produced microscopic lesions in certain tissues at levels as low as 438 ppm. Similarly, in the 13-week study, no toxicological effects were evident in rats exposed up to 7,000 ppm isoprene for 13 weeks. Conversely, hematological and histopathological changes were observed in mice at exposures of 700 ppm and higher. This 13-week repeated dose inhalation study confirmed the species difference between rats and mice in susceptibility to isoprene. Again, in the 26-week repeated dose study, there were no treatment related mortality, bodyweight changes nor laboratory findings in rats. The only treatment related effect was an increased incidence and severity of interstitial cell hyperplasia of the testis at 7,000 ppm (highest dose) compared with controls. However, this lesion which was seen in all recovery groups also occurred at a high incidence in the controls and there was no concentration related trend. In mice exposed to isoprene for 26 weeks, there was reduced survival at the high dose from 18 weeks onwards. Mice exposed for 26 weeks and allowed to recover with no exposure for an additional 26 weeks showed evidence of spinal cord degeneration. After 26 weeks of exposure, spinal cord degeneration was evident only in mice exposed to 7,000 ppm. However, in recovery group animals after a further 26 weeks

without exposure, spinal cord degeneration occurred with a statistically significant increased incidence above control in mice exposed to 70 ppm and above. In the chronic inhalation study (i.e., 80 weeks) conducted in mice, no clinical signs of toxicity were seen other than those associated with tumor development. Of note, in sharp contrast to what was observed in the 26-week study, there was no evidence of spinal cord degeneration in mice in the chronic 80-week study. This discrepancy was further evaluated in 2001 when Dr. Robert Garman, a veterinary pathologist, conducted a neuropathology review of the slides from the 26-week repeat dose study in mice. This review confirmed the presence of minimal to mild degenerative changes within the sections of spinal cord from mice in the highest dose group, i.e., 7,000 ppm that had been necropsied immediately after the six month exposure period. However, no significant differences were found between the appearance of the spinal cord sections from the mice in the 0 ppm; 70 ppm; 220 ppm; and 7,000 ppm groups that were necropsied immediately after the six month recovery period. This review supports the likelihood of recovery of the mice from the mild degenerative lesions found immediately after the six month isoprene exposure. Confirmation of this finding by the NTP is still pending.

2-Methyl-2-butene was tested in a 28-day repeated dose study in rats. Although some general effects were observed in this study, these effects were slight and were most apparent in those animals exposed to the highest dose, i.e., 7,000 ppm, and to a lesser extent to those exposed to 2,000 ppm.

The Pyrolysis C5 stream was also tested in a 28-day repeated dose study in rats at concentrations up to 1,000 ppm. No general systemic effects were observed during the routine clinical examination or in the functional observational battery. Slight histopathological changes were observed in the livers of the high dose rats. In the male rats, however, higher kidney weights and incidence of cortical tubules with hyaline droplets were apparent in all treatment groups. High dose males also showed associated kidney lesions. No pathological changes were apparent in the kidneys of treated females. The kidney effects observed in male rats is a well studied phenomenon known as "light hydrocarbon nephropathy." This phenomenon has been extensively evaluated by the US Environmental Protection Agency (EPA). The EPA has determined that "light hydrocarbon nephropathy" is a male rat-specific phenomenon and has no relevance for human risk assessment.

The Hydrotreated C5 stream was tested in a 28-day repeated dose study in rats at concentrations up to 8,502 ppm. Other than lethargy and increased salivation observed in the high dose animals, i.e., 8,502 ppm, no other treatment-related clinical signs were noted. In addition, no treatment-related changes were observed in motor activity or in the functional observational battery. No macroscopic changes were noted in any of the animals. However, male rats in all treatment groups again showed evidence of "light hydrocarbon nephropathy." Other effects included some minimal changes in the livers of high dose male rats, some atrophy/disorganization of the olfactory epithelium in the nasal turbinates in both high dose males and females as well as mid-dose females and an increased incidence of basophilic cortical tubules in all male exposure groups, and in the female mid and high dose groups compared to the air control groups.

Both *in vitro* and *in vivo* mutagenicity studies have been conducted on the major components and two complex streams from the C5 Non-cyclics Category including isoprene, 2-methyl-2-butene, Pyrolysis C5s stream, and Hydrotreated C5s stream. Isoprene, 2-methyl-2-butene, Pyrolysis C5s stream, and Hydrotreated C5s stream were not genotoxic in any of the *in vitro* assays conducted. However, both isoprene and 2M2B produced positive responses *in vivo*. Exposure of B6C3F1 mice to isoprene resulted in a statistically significant increase in sister chromatid exchanges and bone marrow micronuclei. However, isoprene did not produce an increase in micronucleated lung fibroblasts in exposed F344 rats.

2M2B was also mutagenic at high exposure concentrations (at exposures greater than 3,207 ppm (9,199 mg/m³) when tested *in vivo* for its ability to induce micronuclei in bone marrow

polychromatic erythrocytes (PCEs) in both mice and rats. The Pyrolysis C5s and Hydrotreated C5s streams were not mutagenic either *in vitro* or *in vivo*. The Pyrolysis C5s stream contained 18% isoprene and 3% 2MB2 while the Hydrotreated C5 stream contained 8% 2MB2 and 7% 1,3-butadiene. Results from these two mixed streams suggest that complex streams containing less than 21% isoprene and 2MB2 combined would not likely be mutagenic. Streams containing substantially higher amounts of isoprene and/or 2MB2, such as, the Isoprene Concentrate and perhaps the Isoprene-Piperylene Concentrate, while untested, may have potential for mutagenic activity in the mouse micronucleus assay.

Chronic carcinogenicity studies have only been conducted with isoprene. In these studies there is clear evidence of carcinogenicity of isoprene in mice. Isoprene produced exposure-related increases in the incidence of malignant neoplasms in the liver, lung, Harderian gland and forestomach of mice, as well as increases in the number of hemangiosarcomas and histiocytic sarcomas. In rats, on the other hand, there were no significant increases in the incidence of malignant tumors. In rats isoprene exposures were associated with increases in the rates of benign tumors in the testes and kidney (male) and mammary gland (male and female). Although single incidences of several rare brain neoplasms were observed in female rats, the fact that they were of several distinct cell types, makes it difficult to determine if they are truly exposure related. Of note, due to the high probability that the toxicities seen for isoprene-exposed rodents derive from the formation of diepoxides, and the fact that the mouse has been shown to produce much higher amounts of diepoxide than the rat, the best predictive model for human health would be the species that exhibits the greatest similarity to metabolic alteration of this chemical, i.e., the rat.

No guideline reproductive studies have been conducted with isoprene. However, histopathology of the reproductive organs was evaluated in 13- and 26-week repeated dose inhalation studies conducted in F344 rats and B6C3F1 mice at target concentrations up to 7,000 ppm for 6 hours/day, 5 days/week for 13 weeks (Melnick *et al.*, 1994). Sperm motility and vaginal cytology evaluations were also performed on all rats and mice exposed to 0; 70; 700; or 7,000 ppm of isoprene in the 13-week study. No treatment-related effects were observed in rats exposed to isoprene for 13 weeks. Following 26 weeks of exposure, the only effect in rats was an increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7,000 ppm group. Following the 26 week recovery period, the incidence of interstitial cell adenoma was slightly greater in male rats exposed to 700 ppm or higher. Thus, 220 ppm was selected as the NOAEL for reproductive toxicity in rats. In mice, significant effects were observed at 700 ppm or higher, including increased estrous cycle length and testicular atrophy, decreased epididymal weight, sperm head count, sperm concentration, and sperm motility. Thus, 70 ppm was determined to be the NOAEL for reproductive effects in mice.

A developmental toxicity study was conducted with isoprene in both rats and mice. In this study, isoprene did not produce any maternal or developmental toxicity in rats. However, both maternal and developmental toxicity were evident in mice. In mice, maternal weight gain and uterine weight were significantly reduced at the highest dose (i.e., 7,000 ppm). Significant reductions in fetal bodyweight were observed at the 280 ppm dose level for female fetuses and at the 1,400 ppm level for male fetuses. Thus, in this study, 1,400 ppm was the NOAEL for maternal toxicity. A NOAEL for developmental toxicity could not be determined as effects were observed at the lowest exposure concentration tested (280 ppm).

OECD 422 combined repeated dose and reproduction/developmental toxicity studies were conducted on 2M2B and the Pyrolysis C5 and Hydrotreated C5 streams. In all cases, exposure of rats to these materials did not produce any evidence of reproductive or developmental toxicity at any of the doses tested.

In summary, the above isoprene studies demonstrate a clear species difference between rats and mice with respect to both reproductive and developmental toxicity with the mouse being the more

sensitive species. This species difference is thought to be due primarily to differences in the rates of formation and/or deactivation of the biological active diepoxide, with the mouse producing higher levels. Species comparisons suggest that the rat is a more appropriate model for predicting human risk. Based on these findings, the C5 Non-cyclics Category streams, in particular those with low isoprene content, are considered as having minimal potential to produce reproductive and developmental effects.

This human health assessment for the C5 Non-cyclics Category has been based upon data that include considerable attention towards the most active component (isoprene) of the streams. This most active status was assigned based upon the fact that it is a diolefin, it is known to result in a metabolite that is highly mutagenic (a diepoxide), and that it exhibits higher toxicity than other streams, particularly in the mouse. As the mixed streams Pyrolysis C5s and Hydrotreated C5s (with lesser amounts of isoprene but greater levels of other category components) were also tested, it is highly likely that the toxicities and environmental effects for the category are encompassed within the reported database and assessment.

5 HAZARDS TO THE ENVIRONMENT

5.1 Aquatic Toxicity

The aquatic toxicity of streams in this category is expected to fall within a relatively narrow range regardless of their composition. This is expected because the constituent chemicals of these streams 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, 1995), and the differences between toxicities (i.e., LC/LL₅₀, EC/EL₅₀) 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 streams in this category, supports a critical body residue (CBR) for these chemicals between approximately 2 to 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 umol/g of lipid (Di Toro *et al.*, 2000). Therefore, only hydrocarbon streams 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 umol of hydrocarbon per gram of lipid will demonstrate lethality.

Fish, invertebrate, and alga toxicity data are available for two different complex streams, Pyrolysis C5s and Hydrotreated C5s, and two pure substances, isoprene and 2-methyl-2-butene. These data were applied to characterize the remaining untested streams in this category (Table 16). The 96-hour LC₅₀ results for rainbow trout range between 5.0 to 8.4 mg/L. The 48-hour EC₅₀ results for a daphnid range between 3.0 to 5.8 mg/L. The 96-hour EC₅₀ results based on biomass and growth rate for a green alga range from 10.1 to >35.2 mg/l, while the 96-hour NOEC results based on biomass and growth rate range between 1.7 to 13.1 mg/L.

Table 16. Summary of Aquatic Toxicity Data for Chemical Constituents and Substances in the C5 Non-cyclics Category

CAS RN and Substance Name	Fish Toxicity (<i>Oncorhynchus mykiss</i>) 96-hour LC ₅₀ (mg/L)	Invertebrate Toxicity (<i>Daphnia magna</i>) 48-hour EC ₅₀ (mg/L)	Alga Toxicity (<i>Pseudokirchneriella subcapitata</i>) 96-hour EC ₅₀ ; 96-hour NOEC (mg/L)
78-79-5 Isoprene	7.4 (HLS, 2003j)	5.8 (HLS, 2003k)	15.5b; 1.7b >35.2r; 6.0r (HLS, 2003l)
513-35-9 2-Methyl-2-Butene	5.0 (HLS, 2004a)	3.8 (HLS, 2004b)	10.1b; 3.6b 13.2r; 7.2r (HLS, 2004c)
68476-55-1 Pyrolysis C5s	8.4 (HLS, 2004d)	4.7 (HLS, 2004e)	11.7b; 3.3b 18.4r; 7.8r (HLS, 2004f)
68602-79-9 Hydrotreated C5s	5.3 (HLS, 2004g)	3.0 (HLS, 2004h)	>25.1b; 12.1b >25.1r; 13.1r (HLS, 2004i)

b biomass

r growth rate

HLS Huntingdon Life Sciences Ltd.

Although the isoprene 96-hour EC₅₀ results for growth rate did not establish an effect concentration, a concentration approximately equivalent to the results for biomass, 15.5 mg/L, would be expected. This is supported by results from the Pyrolysis C5s stream that can contain as much as 25% isoprene (Table 2). This stream demonstrated effect concentrations for both endpoints ranging between 11.7 and 18.4 mg/L. The Hydrotreated C5s stream did not establish effect concentrations for both biomass and growth rate. However, the NOEC values for both endpoints were established and were in-line with the NOEC values for the other substances. An estimate of the EC₅₀ ranges for biomass and growth rate for the Hydrotreated C5s stream is 10.1 to 15.5 and 13.2 to 18.4 mg/L, respectively, which is based on the data for isoprene, 2-methyl-2-butene, and the Pyrolysis C5s stream. Although the Hydrotreated C5s stream has been identified as containing 2% isoprene and 11% 2-methyl-2-butene, it has several constituents in common with the Pyrolysis C5s stream (Table 2). The inability for alga testing to consistently identify both the EC₅₀ and NOEC results for chemical constituents and substances in this category is a function of the difficulty in working with materials that are volatile and have relatively low water solubility, as well as the need to work in test systems that are closed with exposure solutions that cannot be renewed as can be accomplished in the fish and daphnid acute test systems.

5.2 Assessment Summary for the Environment

Results of distribution modeling show that streams in the C5 Non-cyclics Category will partition primarily to the air compartment, with a negligible amount partitioning to water. Although constituents have a moderate degree of water solubility, wet deposition of category constituents is not likely to play a significant role in their atmospheric fate because they rapidly photodegrade. Volatilisation to the air will contribute to the rapid loss of category constituents from aqueous and terrestrial habitats. In the air, these constituents have the potential to rapidly degrade through indirect photolytic processes mediated primarily by hydroxyl radicals with calculated degradation

half-lives ranging from 1.2 to 31.8 hours, depending on hydroxyl radical concentration. Aqueous photolysis and hydrolysis will not contribute to the transformation of category constituents in aquatic environments because they are either poorly or not susceptible to these reactions.

Streams and pure chemicals in this category are subject to biodegradative processes. Streams and constituents in this category exhibited a wide range of biodegradability under standard testing procedures, 7 to 61% biodegradation after 28 days. However, the data show that category streams that exhibit lower extents of biodegradation after 28 days can exhibit high extents and rapid rates of biodegradation (>60%) once acclimation occurs. One of the pure chemicals tested, isoprene, which represents one of the category streams and is otherwise found at various concentrations in most of the complex streams, biodegraded to an extent of approximately 60% in 18 days. Overall, the streams from this category are expected to degrade rapidly in the environment from a combination of physical and biological processes.

Aquatic toxicity data exist for two different complex streams and two pure substances in this category. The two complex streams combined contain constituents shared by the remainder of the streams within this category, and therefore justifies their use to characterize the potential effects of the untested streams. This application of read-across data is further supported by data for two pure substances (also constituents of most of the streams in this category), which demonstrated effect values within or very similar to the range of values for the complex substances.

The 96-hour LC₅₀ for *Oncorhynchus mykiss* (rainbow trout) is 5.0 to 8.4 mg/L. The 48-hour EC₅₀ for *Daphnia magna* is 3.0 to 5.8 mg/L. The 96-hour EC₅₀ for *Pseudokirchneriella subcapitata* (green alga) based on biomass and growth rate is 10.1 to 15.5 and 13.2 to 18.4 mg/l, respectively. The 96-hour NOEC based on biomass and growth rate is 1.7 to 12.1 and 6.0 to 13.1 mg/L, respectively.

The aquatic toxicity data suggest that the streams in this category will exhibit a moderate order of toxicity. Most notably, because category constituents possess limited potential to accumulate in aqueous media, the toxicity of category streams towards aquatic species is considered a minimal risk factor in the overall safety assessment of this category.

6 DATA SUMMARY

Physico-chemical, environmental fate and effects, and human health data that characterize the 10 streams in the C5 Non-cyclics Category are summarized in Tables 17 and 18. CAS RNs are associated with streams as follows:

- **Pyrolysis C5s**
 - 68476-55-1
 - 68476-43-7
 - 68527-19-5
 - 68603-00-9
 - 68956-55-8
- **Hydrotreated C5s**
 - 68602-79-9
 - 68410-97-9
 - 68603-00-9
- **Pentenenes**
 - 68476-55-1

- 68527-11-7
- 68603-03-2
- **Piperylene Concentrate**
 - 68477-35-0
 - 64742-83-2
- **Isoprene Concentrate**
 - 68514-39-6
 - 68476-43-7
 - 78-79-5
- **Isoprene-Piperylene Concentrate**
 - 68514-39-6
 - 68476-55-1
- **Isoprene, High Purity**
 - 78-79-5
- **Isoprene Purification Byproduct**
 - 68606-36-0
 - 68476-55-1
- **2-Methyl-2-Butene**
 - 513-35-9
- **Metathesis Byproduct**
 - 68606-29-1

Table 17. Physico-Chemical and Environmental Data Used to Characterize Streams and CAS RNs in the C5 Non-cyclics Category

Endpoint	C5 Non-cyclics Category Streams and CAS RNs									
	Pyrolysis C5s	Hydrotreated C5s	Pentenes	Piperylene Concentrate	Isoprene Concentrate	Isoprene-Piperylene Concentrate	Metathesis Byproduct	Isoprene Purification Byproduct	Isoprene, High Purity	2-Methyl-2-Butene
	68476-55-1, 68476-43-7, 68527-19-5, 68603-00-9, 68956-55-8	68602-79-9, 68410-97-9, 68603-00-9	68476-55-1, 68527-11-7, 68603-03-2	68477-35-0, 64742-83-2	68514-39-6, 68476-43-7, 78-79-5	68514-39-6, 68476-55-1	68606-29-1	68606-36-0, 68476-55-1	78-79-5	513-35-9
Melting Point*/ Range (°C)	-168.5 to -105.5 (m)								-145.9 (m)	-133.7 (m)
Boiling Point*/ Range (°C)	25.0 to 56.5 (m)	23.5 to 52.0 (m)	23.5 to 56.5 (m)					34.0 (m)	38.5 (m)	
Vapor Pressure*/ Range (hPa)	5.85 E2 (m)	8.23 E2 (m)	5.85 E2 to 8.23 E2 (m)					7.33 E2 (m)	6.24 E2 (m)	
Log P _{ow} */ Range	3.19 to 3.25 (m)	2.64 to 4.21 (m)	2.64 to 4.21 (m)					2.42 (m)	2.67 (m)	
Water Solubility*/ Range (mg/L)	49.8 to 423.5 (m)								338.6 (m)	206.1 (m)
Direct Photodegradation	Direct photolysis will not contribute to degradation									
Indirect (OH-) Photodegradation* (half-life, hrs) (c)	1.2 to 31.8 (a)								1.2 (m)	1.5 (m)
Hydrolysis	Hydrolysis will not contribute to degradation									
Distribution*	>99.9% partitions to air <0.1% partitions to water									

* Constituent chemicals used to define selected endpoints except for the Isoprene, High Purity, and 2-Methyl-2-Butene streams include: *cis*-Butene-2, *cis*-Pentene-2, 3-Methyl-1-Butene, 1,4-Pentadiene, Isopentane, Isoprene, n-Pentane, 2-Methyl-2-Butene, Cyclopentene

(m) Measured values

(a) Atmospheric half-life values are based on a 12-hr day

Table 17. Continued

Endpoint	C5 Non-cyclics Category Streams and CAS RNs									
	Pyrolysis C5s	Hydrotreated C5s	Pentenes	Piperylene Concentrate	Isoprene Concentrate	Isoprene-Piperylene Concentrate	Metathesis Byproduct	Isoprene Purification Byproduct	Isoprene, High Purity	2-Methyl-2-Butene
	68476-55-1, 68476-43-7, 68527-19-5, 68603-00-9, 68956-55-8	68602-79-9, 68410-97-9, 68603-00-9	68476-55-1, 68527-11-7, 68603-03-2	68477-35-0, 64742-83-2	68514-39-6, 68476-43-7, 78-79-5	68514-39-6, 68476-55-1	68606-29-1	68606-36-0, 68476-55-1	78-79-5	513-35-9
Biodegradation (% after 28 days)	7.0	11.2 65.4*			7.0 to 11.2 7.0 to 65.4*				60.9**	7.0**
96-hr Fish LC ₅₀ (mg/L)	8.4	5.3			5.0 to 8.4				7.4**	5.0**
48-hr Invertebrate EC ₅₀ (mg/L)	4.7	3.0			3.0 to 5.8				5.8**	3.8**
96-hr Alga EC ₅₀ (mg/L)	11.7b 18.4r	10.1 to 15.5b 13.2 to 18.4r***			10.1 to 15.5b 13.2 to 18.4r				15.5b** 13.2 to 18.4r***	10.1b** 13.2r**
96-hr Alga NOEC (mg/L)	3.3b 7.8r	12.1b 13.1r			1.7 to 12.1b 6.0 to 13.1r				1.7b** 6.0r**	3.6b** 7.2r**

* % biodegradation after 56 days

b biomass

r growth rate

** Data developed on the pure chemical (i.e., isoprene or 2-methyl-2-butene) and not a stream sample

*** A range of values based on read-across from studies in this category is used to characterize the growth rate endpoint because the study did not establish an effect concentration.

Table 18. Human Health Data Summary Used to Characterize Streams and CAS RNs in the C5 Non-cyclics Category

Endpoint	Human Health Data for C5 Non-cyclics Category Streams (CAS RNs)									
	Pyrolysis C5s	Hydrotreated C5s	Pentenes	Piperylene Concentrate	Isoprene Concentrate	Isoprene-Piperylene Concentrate	Metathesis Byproduct	Isoprene Purification Byproduct	Isoprene, High Purity	2-Methyl-2-Butene
	68476-55-1, 68476-43-7, 68527-19-5, 68603-00-9, 68956-55-8	68602-79-9, 68410-97-9, 68603-00-9	68476-55-1, 68527-11-7, 68603-03-2	68477-35-0, 64742-83-2	68514-39-6, 68476-43-7, 78-79-5	68514-39-6, 68476-55-1	68606-29-1	68606-36-0, 68476-55-1	78-79-5	513-35-9
Acute Toxicity (rat)	LC ₅₀ > 61,000 ppm*							LC ₅₀ = 64,620 ppm	LC ₅₀ > 61,000 ppm	
Acute Toxicity (mouse)	LC ₅₀ = 56,363 ppm*							LC ₅₀ = 56,363 ppm	LC ₅₀ = 56,363 ppm*	
Irritation	Mildly irritating to skin, non-irritating to eyes*								Mildly irritating to skin, non-irritating to eyes	
Repeat Dose Toxicity (rat)	NOAEL = 300 ppm	NOAEL = <992 ppm	NOAEL = 300 to 992 ppm*					NOAEL = 2,200 ppm	NOAEL = 580 ppm	
Mutagenicity Ames Assay	Negative*	Negative	Negative*					Negative	Negative	
Mutagenicity Mouse Micronucleus	Negative*	Negative	Negative*	Positive*		Negative*		Positive	Positive	

* Based on read-across data from other members of the category

Table 18. Continued

Endpoint	Human Health Data for C5 Non-cyclics Category Streams (CAS RNs)									
	Pyrolysis C5s	Hydrotreated C5s	Pentenes	Piperylene Concentrate	Isoprene Concentrate	Isoprene-Piperylene Concentrate	Metathesis Byproduct	Isoprene Purification Byproduct	Isoprene, High Purity	2-Methyl-2-Butene
	68476-55-1, 68476-43-7, 68527-19-5, 68603-00-9, 68956-55-8	68602-79-9, 68410-97-9, 68603-00-9	68476-55-1, 68527-11-7, 68603-03-2	68477-35-0, 64742-83-2	68514-39-6, 68476-43-7, 78-79-5	68514-39-6, 68476-55-1	68606-29-1	68606-36-0, 68476-55-1	78-79-5	513-35-9
Reproductive Toxicity (rat)	NOAEL = 1,000 ppm	NOAEL = 8,502 ppm	NOAEL = 1,000 to 8,502 ppm*					NOAEL = 220 ppm	NOAEL = 7,000 ppm	
Developmental Toxicity (rat)	NOAEL = 1,000 ppm	NOAEL = 8,502 ppm	NOAEL = 1,000 to 8,502 ppm*					NOAEL = 7,000 ppm	NOAEL = 7,000 ppm	

* Based on read-across data from other members of the category

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APPENDIX I**ETHYLENE PROCESS DESCRIPTION****A. Ethylene Process****1. Steam Cracking**

Steam cracking is the predominant process used to produce ethylene. Various hydrocarbon feedstocks are used in the production of ethylene by steam cracking, including ethane, propane, butane, and liquid petroleum fractions such as condensate, naphtha, and gas oils. The feedstocks are normally saturated hydrocarbons but may contain minor amounts of unsaturates. These feedstocks are charged to the coils of a cracking furnace. Heat is transferred through the metal walls of the coils to the feedstock from hot flue gas, which is generated by combustion of fuels in the furnace firebox. The outlet of the cracking coil is usually maintained at relatively low pressure in order to obtain good yields to the desired streams. Steam is also added to the coil and serves as a diluent to improve yields and to control coke formation. This step of the ethylene process is commonly referred to as "steam cracking" or simply "cracking" and the furnaces are frequently referred to as "crackers".

Subjecting the feedstocks to high temperatures in this manner results in the partial conversion of the feedstock to olefins. In the simplest example, feedstock ethane is partially converted to ethylene and hydrogen. Similarly, propane, butane, or the hydrocarbon compounds that are associated with the liquid feedstocks are also converted to ethylene. Other valuable hydrocarbon streams are also formed, including other olefins, diolefins, aromatics, paraffins, and lesser amounts of acetylenes. These other hydrocarbon streams include compounds with two or more carbon (C) atoms per molecule, i.e., C₂, C₃, C₄, etc. Propane and propylene are examples of C₃ hydrocarbons and benzene, hexene, and cyclohexane are examples of C₆ hydrocarbons.

2. Refinery Gas Separation

Ethylene and propylene are also produced by separation of these olefins streams, such as from the light ends product of a catalytic cracking process. This separation is similar to that used in steam crackers, and in some cases both refinery gas streams and steam cracking furnace effluents are combined and processed in a single finishing section. These refinery gas streams differ from cracked gas in that the refinery streams have a much narrower carbon number distribution, predominantly C₂ and/or C₃. Thus the finishing of these refinery gas streams yields primary ethylene and ethane, and/or propylene and propane.

B. Products of the Ethylene Process

The intermediate stream that exits the cracking furnaces (i.e., the furnace effluent) is forwarded to the finishing section of the ethylene plant. The furnace effluent is commonly referred to as "cracked gas" and consists of a mixture of hydrogen, methane, and various hydrocarbon compounds with two or more carbon atoms per molecule (C₂+). The relative amount of each component in the cracked gas varies depending on what feedstocks are cracked and cracking process variables. Cracked gas may also contain relatively small concentrations of organic sulfur compounds that were present in the feedstock or were added to the feedstock to control coke formation. The cracked gas stream is cooled, compressed and then separated into the individual streams of the ethylene process. These streams can be sold commercially and/or put into further steps of the process to produce additional materials. In some ethylene processes, a liquid fuel oil product is produced when the cracked gas is initially cooled. The ethylene process is a closed process and the products are contained in pressurized systems.

The final products of the ethylene process include hydrogen, methane (frequently used as fuel), and the high purity products ethylene and propylene. Other products of the ethylene process are typically mixed streams that are isolated by distillation according to boiling point ranges. It is a

subset of these mixed streams that make up the constituents of the C5 Non-cyclics Category. Categories sponsored by the Olefins Panel of the American Chemistry Council are listed in Table 19.

Table 19. HPV Program Categories Sponsored by the Olefins Panel of the American Chemistry Council

Category Number	Category Name
1	Crude Butadiene C4
2	Low 1,3-Butadiene C4
3	C5 Non-cyclics
4	Propylene Streams
5	High Benzene Naphthas
6	Low Benzene Naphthas
7,8,9	Resin Oils & Cycloidiene Dimer Concentrates
10	Fuel Oils
11	Pyrolysis C3+ and Pyrolysis C4+

The chemical process operations that are associated with the process streams in the C5 Non-cyclics Category are shown in Figure 3

Figure 3. C5 Non-cyclics Process Streams Flow Diagram from the Ethylene Manufacturing Process Unit

HPV C5 Non-Cyclics Category streams are shown in bold and with "*" following the name

