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Elizabeth_Moran@americanchemistry.com on 09/20/2001 06:53:08 PM

To: Oscar Hernandez/DC/USEPA/US@EPA
cc: Charles Auer/DC/USEPA/US@EPA, Richard Hefter/DC/USEPA/US@EPA, NCIC OPPT/DC/USEPA/US@EPA, hpv.crtk@epamail.epa.gov, Rtk Chem/DC/USEPA/US@EPA

Subject: Olefins Panel Revised Test Plan for C5 Noncyclics

On November 6, 2000, the Olefins Panel of the American Chemistry Council submitted a test plan and robust summaries on the C5 Noncyclic category. On March 30, 2001, the Panel received comments from EPA regarding the test plan and robust summaries. Attached are a revised test plan and revised robust summaries. Members of the Olefins Panel are listed on page vi of the test plan.

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The Panel has revised the test plan to:

- Remove neohexene (CAS number 558-37-2) from the category,
- Clarify the basis for mammalian and environmental endpoints,
- Address mechanisms for both mammalian and environmental endpoints,
- Indicate that, while physicochemical (PC) data will be calculated as described in EPA guidance documents, available PC data will also be provided for representatives of the category, and measured values will be developed for two products.
- Expand the discussion of the basis for the category to clarify the Panel's intent. The Plan did not propose basing the category analysis on the genotoxicity of isoprene, but, rather, indicated that the data for isoprene and other components of the category streams show that the only adverse health effect likely to be seen in the SIDS battery of tests is genetic toxicity, and
- Include testing of isoprene for acute fish and daphnia toxicity and biodegradation.

The Panel has also included robust summaries for some additional materials, revised some robust summaries to respond to EPA comments, and revised one robust summary on isoprene to add some additional information.

In preparing this test plan, the Panel has given careful consideration to the principles contained in the letter EPA sent to all HPV Challenge Program participants on October 14, 1999. As requested by EPA in that letter, the Panel has sought to maximize the use of scientifically appropriate categories of related chemicals and of structure activity relationships. Additionally, and also as requested in EPA's letter, in analyzing the adequacy of existing data, the Panel has conducted a thoughtful, qualitative analysis rather than use a rote checklist approach. The Panel has taken the same thoughtful approach when developing this revised test plan and believes it conforms to those principles.

(See attached file: Cover Letter.pdf)(See attached file: C5 Noncyclics Test Plan 092001.pdf)(See attached file: Rev Robust Sum ISOPRENE 0907601.pdf)(See attached file: Revised Robust Summaries Isoamylene.pdf)(See attached file: Robust summary Alkenes C6 Rich - Fish Acute 4-23-01.pdf)(See attached file: Robust Summary n-Pentane - Fish Acute 6 -25-01.pdf)

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Robust summary Alkenes C6 Rich - Fish Acute 4-23-01.pdf Robust Summary n-Pentane - Fish Acute 6 -25-01.pdf

September 18, 2001

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Oscar Hernandez, Director
Risk Assessment Division
U.S.Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, DC 20460

RE: Olefins Panels Revised Test Plan for C5 Noncyclic Category Under the HPV Program, HPV Registration No.

Dear Mr. Hernandez:

On November 6, 2000, the Olefins Panel of the American Chemistry Council submitted a test plan and robust summaries on the C5 Noncyclic category. On March 30, 2001, the Panel received comments from EPA regarding the test plan and robust summaries. Attached are a revised test plan and revised robust summaries. Members of the Olefins Panel are listed on page vi of the test plan.

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- Indicate that, while physicochemical (PC) data will be calculated as described in EPA guidance documents, available PC data will also be provided for representatives of the category, and measured values will be developed for two products.
- Expand the discussion of the basis for the category to clarify the Panel's intent. The Plan did not propose basing the category analysis on the genotoxicity of isoprene, but, rather, indicated that the data for isoprene and other components of the category streams show that the only adverse health effect likely to be seen in the SIDS battery of tests is genetic toxicity, and
- Include testing of isoprene for acute fish and daphnia toxicity and biodegradation.

The Panel has also included robust summaries for some additional materials, revised some robust summaries to respond to EPA comments, and revised one robust summary on isoprene to add some additional information.

In preparing this test plan, the Panel has given careful consideration to the principles contained in the letter EPA sent to all HPV Challenge Program participants on October 14, 1999. As requested by EPA in that letter, the Panel has sought to maximize the use of scientifically appropriate categories of related chemicals and of structure activity relationships. Additionally, and also as requested in EPA's letter, in analyzing the adequacy of existing data, the Panel has conducted a thoughtful, qualitative analysis rather than use a rote checklist approach. The Panel has taken the same thoughtful approach when developing this revised test plan and believes it conforms to those principles.

If you have any questions, please call me at (301) 924-2006.

Yours truly,

Elizabeth J. Moran, Ph.D.
Manager, Olefins Panel

cc: C. Auer, EPA
R. Hefter, EPA

AR 201 - 13186A

HIGH PRODUCTION VOLUME (HPV)

CHEMICAL CHALLENGE PROGRAM

REVISED

TEST PLAN

For The

C5 Non-Cyclics Category

Prepared by:

American Chemistry Council
Olelins Panel
HPV Implementation Task Group

September 20, 2001

PLAIN ENGLISH SUMMARY

This category test plan addresses ten petrochemical streams (products) derived from the ethylene and associated C5 manufacturing processes. The category has been designated "C5 Non-Cyclics". Streams from this category include complex mixtures containing primarily C5 and C6 alkane, alkene, alkyne, and di-alkene chemicals. Some of these streams may contain as many as 50 chemical components. Because of the complexity of the ten streams, it is appropriate to subcategorize these streams into a lesser number based upon similarities or trends in the percentages of chemical constituents. Further, the unsaturation (double bonds) found in chemicals from these streams is an important parameter for projecting toxic potentials, and thus, streams containing disparate degrees of unsaturation are nominated for testing to provide a full range of information on the toxic potential for this HPV category. Based upon existing information plus newly to-be-developed data, scientifically based characterizations of all streams can be achieved.

Human Health Effects

Based on examination of existing data for components of the streams in the C5 Non-Cyclics category, isoprene and, to a lesser extent, 2-methyl-2-butene are expected to be major contributors to toxicological activity in the SIDS battery of human health tests, with genotoxicity the effect most likely to be seen.

The strategy of this screening level test plan for characterizing human health hazards of members of this category is to evaluate data for numerous components of the streams (existing data and new data that will be generated by the Olefins Panel and by other groups as part of the EPA HPV, OECD SIDS, and ICCA HPV programs), and for two representative mixed streams tested in this program. These data are expected to be sufficient to adequately characterize the human health hazards of the substances included in this category.

The following human health tests will be conducted by the Olefins Panel within this test plan:

- Bacterial genetic toxicity tests, mouse inhalation genetic toxicity tests, and rat inhalation repeated dose tests for toxicity to the reproductive, developmental, and nervous systems with two streams representing mid (Pyrolysis C5s) and low levels (Hydrotreated C5s) of diolefins including isoprene plus two different combinations of the other components (alkanes & alkenes) present in the streams in the C5 Non-Cyclics category.

- Rat inhalation repeated dose test for toxicity to the reproductive, developmental, and nervous systems with a high purity 2-methyl-2-butene stream. 2-Methyl-2-butene is also sponsored by the Olefins Panel in the ICCA HPV Program.

Ecotoxicity, Environmental Fate, and Physical Chemical Properties

Based on existing data and computer modeling for chemical components, the product streams in this category are expected to exhibit a narrow range of aquatic toxicity. For the endpoints in this testing program, all chemical components of this category are expected to act by a similar mode of toxicity, non-polar narcosis. To confirm that the various streams in this category exhibit comparable toxicity in the three aquatic organisms included in HPV testing, the Panel will test two streams, Pyrolysis C5s and Hydrotreated C5s, representing two different combinations of components present in the streams from the C5 Non-Cyclics category. Additional testing will include two high purity substances, isoprene and 2-methyl-2-butene, which are contained in the streams in this category. Isoprene and 2-methyl-2-butene are also sponsored by the Olefins Panel in the ICCA HPV Program.

The testing for each of these substances will include ***an*** alga toxicity test, a ***Daphnia*** sp. acute toxicity test, and a fish acute toxicity test.

One stream (Metathesis Byproduct) in this category contains a higher proportion of C6 chemicals and may produce slightly greater toxicity than the streams and chemicals identified for testing above. To characterize the aquatic toxicity of the Metathesis Byproduct stream, that contains 5 1% C6 chemicals, existing acute fish data for a C6 chemical product (C6 alkenes, a mixture of C6 branched and linear internal olefins) will be applied. In addition, daphnid and alga toxicity data for the same C6 product will be made available to the Olefms Panel through a testing program committed to by the Higher Olefins Panel of the American Chemistry Council. These data, in combination with results from the studies conducted for the Pyrolysis C5s stream, Hydrotreated C5s stream, isoprene, and 2-methyl-2-butene, will characterize the potential toxicity of all products in this category.

To investigate biodegradability of products in this category, the Panel will test two representative mixed streams (Pyrolysis C5s and Hydrotreated C5s) and 2-methyl-2-butene. Results from the proposed testing, in conjunction with existing data for component chemicals, will adequately characterize the potential biodegradability of product streams in this category.

Predictive computer models will be used to develop relevant environmental fate and physicochemical data for chemicals in the C5 Non-Cyclics category. Environmental fate information will be developed either through the use of computer models or in technical discussions when data are lacking or computer modeling is not practical. For mixed streams, physicochemical properties will be represented as a range of values according to component composition. These data will be calculated using a computer model cited in an EPA guidance document prepared for the HPV Program. In addition, measured physicochemical data will be identified for selected product streams in this category. The use of computer modeling for the development of these data is justified since components of the streams in this category are all chemically related and are expected to exhibit minimally different environmental properties based upon the minor structural variations between these chemicals.

EXECUTIVE SUMMARY

The Olefins Panel (Panel) of the American Chemistry Council and the Panel's member companies hereby submit for review and public comment the test plan for the "C5 Non-Cyclics" category under the Environmental Protection Agency's (EPA) High Production Volume (HPV) Chemical Challenge Program. It is the intent of the Panel and its member companies to use new information in conjunction with a variety of existing data and scientific judgment/analyses to adequately characterize the SIDS (Screening Information Data Set) human health, environmental fate and effects, and physicochemical endpoints for this category.

This category test plan addresses ten petrochemical streams (products) derived from the ethylene and associated C5 manufacturing processes. The category includes complex mixtures containing primarily C5, and C6 alkane, alkene, alkyne, and di-alkene chemicals. Some of these products may contain as many as 50 chemical components. All but two of these streams contain isoprene. One of the streams without isoprene contains 93% 2-methyl-2-butene and 7% 2-methyl- 1 -butene, which are components of other streams within the category. The other stream not containing isoprene is Metathesis Byproduct. The pentenes and 2-butene found in the Metathesis Byproduct stream are components of other streams within the category. Because of the complexity of the ten streams, it is appropriate to subcategorize these streams into a lesser number based upon similarities or trends in the percentages of chemical constituents. Further, the unsaturation (double bonds) found in chemicals from these streams is an important parameter for projecting toxic potentials, and thus, streams containing disparate degrees of unsaturation are nominated for testing to provide a full range of information on the toxic potential for this HPV category.

The streams and chemicals that will be tested by the Olefins Panel are described below:

- Pyrolysis C5s: This is a mid-range isoprene stream typically containing 14-20% isoprene, significant amounts of other C5 dienes (e.g., cyclopentadiene and pentadienes), dicyclopentadiene, and most of the C5 mono-olefins (including 1-5% 2-methyl-2-butene) that are present in the other streams in the C5 Non-Cyclics Category.
- Hydrotreated C5s: This is a low isoprene stream typically containing approximately 2% isoprene and very small amounts of other C5 dienes; no dicyclopentadiene; substantial concentrations of pentenes, pentanes, and cyclopentene; and $\leq$11% 2-methyl-2-butene.
- Isoprene (high purity).
- 2-Methyl-2-Butene (high purity).

Based upon existing information plus the newly to-be-developed data from this and other testing programs, scientifically based characterizations of all streams can be achieved.

Human Health Effects

The following human health tests will be conducted by the Olefins Panel within this test plan:

- Pyrolysis C5s and Hydrotreated C5s: Ames tests (OECD Guideline 471), mouse inhalation micronucleus tests (OECD Guideline 474), and rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screens (OECD Guideline 422).

- 2-Methyl-2-Butene: A rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422). 2-Methyl-2-butene is also sponsored by the Olefins Panel in the ICCA HPV Program.

Based upon examinations of stream compositions and existing toxicity data for components of streams in the C5 Non-Cyclics category, there is minimal likelihood for the appearance of unexpected or remarkable biological findings in testing of streams within this chemical class. Chemicals in this category have been extensively observed relative to their impact on exposed humans as well as having been subjected to thorough assessments for CNS (central nervous system) depressant activities and anesthetic potencies, irritation properties, and cardio-sensitization potentials. Reviews of this literature appear in *Patty's Industrial Hygiene & Toxicology chapters 19 & 20, volume IIB, 4th edition* **(I 994).**

Adverse effects from airborne exposures only are considered in this test program. The properties of exposures to liquid hydrocarbons are well known, and include mild dermatitis and a potential to cause chemical pneumonitis if ingested.

Due to the unsaturation within these stream components, isoprene and, to a lesser extent, 2-methyl-2-butene are expected to be major contributors to toxicological activities in endpoints included in the SIDS battery of tests in this chemical category. This expectation is supported by existing data for components of the streams in this category.

The strategy of this screening level test plan for characterizing the human health hazards of the members of this category is to evaluate data for two representative mixed streams and for several of the components of the streams (existing data and new data that will be generated by the Olefins Panel and by other groups as part of the EPA HPV, OECD SIDS, and ICCA HPV programs). These data are expected to be sufficient to adequately characterize the human health hazards of the substances included in this category.

Ecotoxicity, Environmental Fate, and Physical Chemical Properties

Based on existing data for structural analogs and results of computer modeling for selected chemical components, a narrow range of aquatic toxicity is expected for the streams in the category. This is expected because the chemical components in the streams fi-om this category act by a similar mode of toxicity, non-polar narcosis. To confirm that the various streams in this category are similarly toxic to the three aquatic organisms included in the HPV Program, the Panel will test the following streams and chemicals: Pyrolysis C5s, Hydrotreated C5s, 2-methyl-2-butene, and isoprene (2-methyl-2-butene and isoprene are also sponsored by the Olefms Panel in the ICCA HPV Program).

The testing for all these materials will include an alga toxicity test (OECD Guideline 201), a *Daphnia* sp. acute toxicity test (OECD Guideline 202), and a fish acute toxicity test (OECD Guideline 203).

To adequately characterize the aquatic toxicity of the Metathesis Byproduct stream, which contains 5 1% C6 chemicals, existing acute fish data for a C6 branched and linear alkenes product will be used, along with daphnid and alga toxicity data for the same C6 alkenes product which will be made available to the Olefins Panel through a testing program committed to by the Higher Olefins Panel of the American Chemistry Council. These data, in combination with results from the studies conducted for the Pyrolysis C5s stream, Hydrotreated C5s stream, isoprene, and 2-methyl-2-butene, will characterize the potential toxicity of all products in this category.

To investigate the biodegradability of products in this category, the Panel will test, via manometric respirometry (OECD Guideline 301F), two representative mixed streams (Pyrolysis C5s and Hydrotreated C5s) and 2-methyl-2-butene. Results from the proposed testing, in conjunction with existing data for component chemicals, will adequately characterize the potential biodegradability of product streams in this category.

Predictive computer models will be used to develop relevant environmental fate and physicochemical data for chemicals in the C5 Non-Cyclics category. For mixed streams, physicochemical properties will be represented as a range of values according to component composition, and calculated using a computer model cited in an EPA guidance document prepared for the HPV Challenge Program. In addition, measured physicochemical data will be identified for selected product streams in this category. The use of computer modeling for the development of these data is justified since components of the streams in this category are all chemically related and are expected to exhibit minimally different environmental properties based upon the minor structural variations between these chemicals.

LIST OF MEMBER COMPANIES
THE OLEFINS PANEL

The Olefins Panel includes the following member companies:

ATOFINA Petrochemicals, Inc.*
BP Amoco Chemical Company
Chevron Phillips Chemical Company
CONDEA Vista Company*
The Dow Chemical Company
E. I. du Pont de Nemours and Company*
Eastman Chemical Company*
Equistar Chemicals, LP
ExxonMobil Chemical Company
Formosa Plastics Corporation, U.S.A.*
The B.F.Goodrich Company*
The Goodyear Tire & Rubber Company
Huntsman Corporation
Koch Industries*
NOVA Chemicals Inc.
Shell Chemical Company
sunoco, Inc.*
Texas Petrochemicals Corporation*
Westlake Chemical Corporation*
Williams Olefins, LLC*

* These companies are part of the Olefins Panel but do not produce streams in the C5 Non-Cyclics Category.

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REVISED TEST PLAN FOR THE C5 NON-CYCLICS CATEGORY

I. INTRODUCTION

The Olefins Panel (Panel) of the American Chemistry Council and the Panel's member companies have committed to develop screening level human health effects, environmental effects and fate, and physicochemical data for the C5 Non-Cyclics category under the Environmental Protection Agency's (EPA's) High Production Volume (HPV) Challenge Program (Program).

In preparing this test plan, the Panel has given careful consideration to the principles contained in the letter EPA sent to all HPV Challenge Program participants on October 14, 1999. As directed by EPA in that letter, the Panel has sought to maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships. Additionally, and also as directed in EPA's letter, in analyzing the adequacy of existing data, the Panel has conducted a thoughtful, qualitative analysis rather than use a rote checklist approach. The Panel has taken the same thoughtful approach when developing its test plan. The Panel believes its test plan conforms to the principles articulated in EPA's letter.

This plan identifies CAS numbers used to describe process streams in the category, identifies existing data of adequate quality for substances included in the category, and outlines testing needed to develop screening level data for this category under the Program. This document also provides the testing rationale for the C5 Non-Cyclics category. The objective of this effort is to identify and develop sufficient test data and/or other information to adequately characterize the human health and environmental effects and environmental fate for the category in accordance with the EPA HPV Program. Physicochemical data that are requested in this program will be calculated as described in EPA guidance documents. In addition, measured data will be provided for selected products in this category when available.

II. DESCRIPTION FOR THE C5 NON-CYCLICS CATEGORY

A. The Category

The C5 Non-Cyclics category was developed for the HPV program by grouping ethylene manufacturing streams that exhibit commonalities from both manufacturing process and compositional perspectives. The fifteen CAS numbers listed in Table 2 each represent a production stream. However, these 15 streams are narrowed to 10 as certain streams are identified using more than one CAS number. Eight of these process streams are complex products containing many components. CAS numbers are used to represent these eight stream products, but are assigned vague verbal descriptions for distinguishing the streams. Certain single streams are correctly represented by more than one CAS number, and a CAS number may be applicable to more than one stream. A description of the ethylene and associated stream production processes is included in Appendix I.

The streams in this category consist of high purity hydrocarbons or complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes (with the exception of the Metathesis Byproduct stream which has 5 1% hexenes) and predominantly non- cyclic. The typical compositions of the streams are shown in Table 3. All but two of these streams contain isoprene. One of the streams without isoprene contains 93% 2-methyl-2-butene and 7% 2-methyl- 1 -butene, which are components of other streams within the category. The other stream not containing isoprene is Metathesis Byproduct which contains 44% pentenes, 51% hexenes, and 3% 2-butene. The pentenes and 2-butene found in the Metathesis Byproduct stream are components of other streams within the category. Typically, only five of the many components of the streams (isopentane, isoprene, pentane, 2-methyl-2-butene, 1,3-pentadiene) are present at concentrations $\geq$30%; and only six more components (2-butene, isopentene, 2-pentene, cyclopentadiene, cyclopentene, methyl-pentenes-2) are present at $\geq$20%. The category is designated C5 Non-Cvclics.

The CAS Numbers in the C5 Non-Cyclics category are associated with ten streams that are commercial products or isolated intermediates:

1. Pyrolysis C5s
2. Hydrotreated C5s
3. Pentenes
4. Piperylene Concentrate
5. Isoprene Concentrate
6. Isoprene-Piperylene Concentrate
7. Isoprene, High Purity
8. Isoprene Purification Byproduct
9. 2-Methyl-2-Butene
10. Metathesis Byproduct

Descriptions of the ten streams associated with the C5 Non-Cyclics category are presented below:

1. Pyrolysis C5s

Pyrolysis C5s (or C5 fraction) consist of a hydrocarbon distillate fraction separated from pyrolysis gasoline (the C5+ portion of the cracked gas in the ethylene process). The carbon number distribution of the product is predominantly C5, but the stream also typically contains relatively low levels of the higher boiling C4 substances (e.g. 1,2-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

Hydrotreated C5s result from hydrogenation of Pyrolysis C5s 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 Hydrotreated C5s is very low.

3. Pentenes

Pyrolysis C5s are 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 stream has a carbon number distribution that is predominantly C4-C5, consisting in part of iso-pentane and the more volatile pentenes such as 1 -pentene, with about 1-3% isoprene. The stream typically contains the C4 compounds that were present in the Pyrolysis C5s, including 1,3-butadiene. Alternately, Pentenes can be removed later in processing, for example by distillation of the Isoprene Concentrate.

4. Piperylene Concentrate

Production of Piperylene Concentrate (cis- and trans- 1,3-pentadiene) from Pyrolysis C5s is accomplished 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 distribution for Piperylene concentrate 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

The isoprene-piperylene splitter described for the above stream also yields Isoprene Concentrate as a distillate. The carbon number distribution for Isoprene concentrate is predominantly C5. A typical Isoprene Concentrate stream contains 40% isoprene with the balance largely iso- and n-pentane and C5 monolefms. 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

The intermediate process stream charged to the isoprene-piperylene splitter (as described above for piperylene concentrate) is sometimes isolated as a product. This stream typically contains about 20% isoprene and 14% piperylenes.

7. Isoprene, High Purity

High purity isoprene (98+%) is produced by separation from isoprene concentrate. This is accomplished using an extractive distillation process.

8. Isoprene Purification Byproduct

Isoprene Purification Byproduct is a byproduct from the Isoprene purification process. The carbon number of the stream is predominantly C5 and the composition is largely iso- and n-pentane, plus lesser amounts of pentenes and about 5% isoprene. The byproduct may also contain 1,3-butadiene at about 0.5%.

9. 2-Methyl-2-Butene

The component 2-methyl-2-butene 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

An olefins plant may include a Metathesis process which converts ethylene and/or butenes into propylene. This process produces a byproduct (referred to here as Metathesis Byproduct). The stream is a gasoline stream consisting primarily of C5 and C6 olefins.

III. TEST PLAN RATIONALE

A. Human Health Effects - Overview

In addition to a nearly complete SIDS data set for isoprene and genetic toxicity data for 2-methyl-2-butene, a substantial amount of toxicity data are available for many of the other components of the streams in the C5 Non-Cyclics category. Some of the components are SIDS materials, and some components will be tested by the American Chemistry Council Olefins Panel within other category test plans or by other groups within the EPA or ICCA HPV programs.

Based upon examinations of stream compositions and existing toxicity data for components of streams in the C5 Non-Cyclics category, there is minimal likelihood for the appearance of unexpected or remarkable biological findings in testing of streams within this chemical class. Chemicals in this category have been extensively observed relative to their impact on exposed humans as well as having been subjected to thorough assessments for CNS depressant activities and anesthetic potencies, irritation properties, and cardio-sensitization potentials. Reviews of this literature appear in Patty *'s Industrial Hygiene & Toxicology, chapters 19 & 20, volume IIB, 4th edition (1994).*

Adverse effects from airborne exposures only are considered in this test program. The properties of exposures to liquid hydrocarbons are well known, and include mild dermatitis and a potential to cause chemical pneurnonitis if ingested.

The mono-olefins possess slightly greater toxic activities than the corresponding alkanes. Hydrocarbons with one double bond are also subject to oxidative metabolism. These metabolites may exhibit weak mutagenic activity (eg., 1,2-butylene oxide), but otherwise have similar toxic profiles to comparable alkanes. There are 8 diolefins known within these streams. Two of these (1,3-butadiene and isoprene) are known to exhibit greater toxicity (mutagenicity and tumorigenicity) in species that oxidize the chemicals to diepoxides, i.e., mice > rats. These diepoxides also demonstrate greater activity in cell culture assays for mutagenicity. These observations and logic suggest that diolefins possess greater toxic potential than the corresponding mono-olefins or alkanes, thus providing the rationale for the presumption that, of the components in these streams, the diolefins are the most reactive from a biological viewpoint.

Due to the unsaturation within these stream components, isoprene and, to a lesser extent, 2-methyl-2-butene are expected to be major contributors to toxicological activities in the SIDS battery of tests in this chemical category. This expectation is supported by existing data for components of the streams in the C5 Non-Cyclics category. Genotoxicity is the effect most likely to be seen. Of the SIDS human health endpoints, only the genetic toxicity tests are known to show a dose-related adverse response with isoprene. In a 13 week subchronic rat inhalation toxicity study which included reproductive and developmental toxicity endpoints, isoprene produced no exposure-related effects except slight changes in the testis at the highest exposure level (7000 ppm). With the exception of acute central nervous system effects at high concentrations, none of the other components that are present in substantial amounts in these streams has demonstrated, in endpoints included in the SIDS battery of tests, a potential to cause significant adverse health effects.

It is possible that the biological activity of isoprene, which is limited to positive genetic toxicity in the SIDS tests, may be observed in streams within this category that contain isoprene. However, since metabolism of isoprene is required for toxicity, and other C5 alkenes are metabolized through a common P450 metabolic pathway, it is anticipated that multiple components will compete for the same active enzyme sites. Component toxicities, which are dependent on the formation of biologically active metabolites, may be reduced as less metabolite(s) will be produced through competition for these sites. Hence the positive genotoxicity of isoprene, or the less potent 2-methyl-2-butene, may in fact be reduced or eliminated by the greater presence of the other components or their biologically-inactive metabolites. This can only be assessed by testing mixed streams.

Thus, the strategy of this screening level test plan for characterizing the human health hazards of this category is to evaluate data for several of the components of the streams and for two representative mixed streams. These data are expected to be sufficient to adequately characterize the toxicity of the substances included in this category.

The details of the strategy are as follows:

1. Two streams representative of the range of compositions found within the mixed streams of the category will be tested. The two streams will contain no single component at a concentration greater than 50%. In order to determine the impact of various levels of isoprene on the mixture, one of the streams will contain a mid-range isoprene content and the other stream will contain a low level of isoprene. The various mixed streams within the category that fit these criteria can be roughly divided into two sets: 1) one containing low levels of C5 mono-olefins (including 2-methyl-2-butene) that are present in the streams of the C5 Non-Cyclics category, but significant amounts of dienes (including isoprene, dicyclopentadiene, and pentadiene) and 2) one containing very small amounts of C5 dienes (including isoprene) and no dicyclopentadiene, but substantial concentrations of pentenes, pentanes, and cyclopentene. Based on these compositional criteria, the following streams were selected for testing:

   a. Pyrolysis C5s

      This stream will be tested to assess the toxicity of streams with a mid-range (approximately 14-20%) isoprene content in addition to significant amounts of other dienes (e.g., cyclopentadiene, pentadienes, and dicyclopentadiene), and most of the C5 mono-olefins that are present in the other streams in the C5 Non-Cyclics Category. The stream will be tested as derived from the production facility, and not as a prepared mixture. The exact composition of the tested stream will be determined analytically at the time of testing. The stream will be tested in a full SIDS human health test battery (except for acute inhalation toxicity which is not deemed informative for the HPV Program). The following tests will be conducted: A bacterial gene mutation test (Ames test, OECD Guideline 47 1), a mouse inhalation micronucleus test for chromosome aberrations (OECD Guideline 474), and a rat inhalation combined repeated dose/reproductive

and developmental effects/neurotoxicity screen (OECD Guideline 422). This strategy will allow an evaluation of the impact of isoprene on the toxicity of the mixed streams, and will also allow an assessment of the hazards of the other components when the influence of isoprene is reduced or eliminated.

b. Hydrotreated C5s

This stream will be tested to assess the toxicity of streams with a low (approximately 2%) isoprene content, small amounts of other C5 dienes, no dicyclopentadiene, substantial concentrations of pentenes, pentanes, cyclopentene, and approximately 11% 2-methyl-2-butene. The test material will be as derived from the production facility. The exact composition of the stream to be tested will be determined analytically at the time of testing. The stream will be tested in a full SIDS human health test battery (except for acute inhalation toxicity which is not deemed informative for the HPV Program). The following tests will be conducted: A bacterial gene mutation test (Ames test, OECD Guideline 471), a mouse inhalation micronucleus test for chromosome aberrations (OECD Guideline 474), and a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422). This strategy will allow an evaluation of the impact of a very low level of isoprene on the toxicity of the mixed streams, and will also allow an assessment of the hazards of the other components when the influence of isoprene is reduced or eliminated.

2. 2-Methyl-2-butene will be tested. This stream is typically 93% 2-methyl-2-butene and 6.7% 2-methyl- 1-butene. 2-Methyl-2-butene is sold as a high-purity material and is also a component in the Pyrolysis C5s, Hydrotreated C5s, Pentenes, Isoprene Concentrate, Piperylene Concentrate, and Isoprene-Piperylene Concentrate streams. The SIDS human health data set (except for the acute inhalation toxicity test which is not deemed informative for the HPV Program) will be completed for 2-methyl-2-butene. Genetic toxicity tests are currently available. 2-Methyl-2-butene, like isoprene, was positive in the mouse chromosome aberration test but negative in the bacterial gene mutation test. A rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422) will be conducted. 2-Methyl-2-butene is also sponsored by the Olefins Panel in the ICCA HPV Program.

3. Evaluation of existing data and new data resulting from other testing programs will be conducted for most of the components present in significant amounts in the streams of the C5 Non-Cyclics category.

a. Existing data: See Table 5.

b. 1 -Butene: Data gaps will be filled under the Olefins Panel's Low Butadiene C4 HPV Test Plan. 1 -Butene is also sponsored by the Olefins Panel in the ICCA HPV Program.

c. 2-Butene: OECD SIDS.

d. n-Pentane: Included in American Petroleum Institute's HPV Test Plan.

e. n-Pentane, isopentane, and cyclopentane: These materials are included in the American Chemistry Council Hydrocarbon Solvents Panel's HPV Test Plan in the C5 Aliphatic Category.

f. 1 -Pentene and isopentene: Although the pentenes are not covered by the American Chemistry Council Higher Olefins Panel's HPV Test Plan based on structural similarity, the data obtained for hexenes within that program can be used for read-across to the pentenes. In addition, data for 1-hexene collected as part of the OECD SIDS program can be used for read-across to 1-pentene.

g. Cyclopentane: Included in American Chemistry Council Hydrocarbon Solvents Panel's HPV program.

h. 1,3-Pentadiene: OECD SIDS.

i. Dicyclopentadiene: OECD SIDS.

j. 1-Hexene, 2-hexene, 3-hexene, methyl-2-pentenes: The American Chemistry Council Higher Olefins Panel will test, as part of the HPV program, a C6 alkenes stream that is comprised of a mixture of branched and linear internal alkenes. Data exist for the C6 alpha olefin, 1-hexene, that was included in an OECD SIDS testing program.

The inhalation route of exposure was chosen for the health effects testing because inhalation is the most relevant route of exposure for the C5 Non-Cyclics streams. The mouse micronucleus test was chosen for chromosomal effects testing because isoprene is negative in *in vitro* tests but positive in the mouse micronucleus test. 2-Methyl-2-butene is also positive in the mouse micronucleus test. The mouse is the standard species for micronucleus tests and a substantial historical database exists for the mouse in this test. The rat will be used in the repeated dose/reproductive and developmental effects/neurotoxicity screen because this test was designed for the rat and there is a historical database for the rat but not for the mouse. The rat is also the standard species for reproductive toxicity tests. Furthermore, there is a substantial amount of data developed in rats, mice, primates, and humans *(in vitro)* providing strong support for the proposition that the rat is a scientifically more appropriate model for humans than is the mouse for the toxicological assessment of diolefins (e.g., butadiene and isoprene).

The recommended testing, together with existing data and data for the components under development by the American Chemistry Council Olefins Panel for other categories under the HPV program, by other HPV consortia, and by the OECD SIDS program, will sufficiently characterize the toxicity of the related hydrocarbon substances included in this category. This position is supported by the fact that previous in-depth testing and human experience with many of the category's components demonstrate a lack of biological activities that are outside of the range of typical alkane/alkene effects.

B. Human Health Effects - Stream Specific Rationales

The rationales for the test plan strategy specific to each stream in the C5 Non-Cyclics category are presented below:

1. Pyrolysis C5s

This stream will be tested in a complete SIDS battery of human health tests (except for acute toxicity) to assess the toxicity of streams with a mid-range (approximately 14-20%) isoprene content in addition to significant amounts of other dienes (e.g., cyclopentadiene, pentadienes, and dicyclopentadiene), and most of the C5 mono-olelins (including 2-methyl-2-butene) that are present in the other streams in the C5 Non-Cyclics category. This strategy will allow an assessment of (1) the impact of isoprene on the toxicity of the mixed streams, (2) the hazards of a subset of the other components when the influence of isoprene is reduced or eliminated, and (3) the hazards of the first of two general groupings of components found in the streams of the C5 Non-Cyclics category.

2. Hydrotreated C5s

This stream will be tested in a complete SIDS battery of human health tests (except for acute toxicity) to assess the toxicity of streams with a low (approximately 2%) isoprene content; small amounts of other C5 dienes; no dicyclopentadiene; substantial concentrations of pentenes, pentanes, cyclopentene; and approximately 11% 2-methyl-2-butene. This strategy will allow an assessment of (1) the impact of a very low level of isoprene on the toxicity of the mixed streams, (2) the hazards of a subset of the other components when the influence of isoprene is reduced or eliminated, and (3) the hazards of the second of the two general groupings of components found in the streams of the C5 Non-Cyclics category.

3. Pentenes

This stream is similar to the Hydrotreated C5s stream in that both streams contain approximately 2% isoprene and are mostly comprised of pentanes and pentenes. Most components of the Pentenes stream are found in the Hydrotreated C5s stream and all components of the Pentenes stream are found in the Pyrolysis C5s stream. With respect to the HPV Program endpoints, the human health toxicity profile of this stream is expected to be the same as that of the Hydrotreated C5s stream. No testing is proposed for this stream at this time. Because the stream is without significant levels of diolefins, there is no basis to project that this stream will exhibit more pronounced or even equal toxicity, qualitatively or quantitatively, to either the Pyrolysis C5 or Hydrotreated streams.

4. Piperylene Concentrate

This stream is similar to the Hydrotreated C5s stream except for the presence of a large amount of 1,3-

pentadiene (typically 30-60%). Two components present at low concentrations in Piperylene Concentrate are absent in Hydrotreated C5s: 2-pentene (1- 10%) and methylpentenes (5%). 2-Pentene is present in the Pyrolysis C5s stream that will be tested. The potential toxicity of methylpentenes will be determined by the testing of a stream containing C6 branched and linear alkenes by the Higher Olefins Panel. The methylpentanes component (16%) present in Piperylene Concentrate is likely to contain 2-methylpentane, which is present at 5% in the Hydrotreated C5s stream that will be tested. 1,3-Pentadiene is an OECD SIDS material and all SIDS endpoints have been adequately addressed. The SIDS Initial Assessment Report (SIAR) indicates that 1,3-pentadiene (cis and trans combined) is of low concern for further testing, and proposes no additional testing. The SIAR indicated that 1,3-pentadiene displayed a low order of acute toxicity, was non-genotoxic, and, in an oral screening study in rats, displayed no systemic lesions, reproductive effects or developmental effects with the NOAEL being 1000 mg/kg, the highest dose tested. The toxicity of the Piperylene Concentrate stream can be characterized by data for 1,3-pentadiene and the Hydrotreated C5s stream.

5. Isoprene Concentrate

This stream is similar to the Pyrolysis C5s stream except that this stream may have a higher concentration of isoprene (14-80%). All except two components of the Isoprene Concentrate stream are present in the Pyrolysis C5s stream. The two components, 3-methyl- 1,2-butadiene and 1,2-butadiene are present at very small concentrations (3.5% and 1.2%, respectively) and are not likely to affect the toxicity profile of the Isoprene Concentrate stream. Depending upon the isoprene concentration, existing data for high purity isoprene or data from testing of the Pyrolysis C5s stream, which has approximately 14-20% isoprene, can be used to characterize the toxicity of Isoprene Concentrate. No additional testing is proposed for this stream at this time.

6. Isoprene-Piperylene Concentrate

This stream, containing 20% isoprene and 14% piperylene, is similar to the Pyrolysis C5s stream. All components of this stream are present in the Pyrolysis C5s stream. Data from testing of the Pyrolysis C5s stream, plus the available toxicity data on isoprene and piperylenes, can be used to adequately characterize the toxicity of Isoprene-Piperylene Concentrate. No additional testing is proposed for this stream at this time.

7. Isoprene, High Purity

Isoprene has been extensively tested and all HPV Chemical Program human health endpoints have been adequately addressed. A significant amount of additional toxicology data is also available. Of the SIDS human health endpoints, only the genetic toxicity tests are known to show a dose-related adverse response with isoprene. In a 13 week subchronic rat inhalation toxicity study which included reproductive and developmental toxicity endpoints, isoprene produced no exposure-related effects except slight changes in the testis at the highest exposure level (7000 ppm). Isoprene is also sponsored by the Olefins Panel in the ICCA HPV Program.

8. Isoprene Purification Byproduct

This stream is predominantly (50-70%) isopentane, which is being addressed by the American Chemistry Council Hydrocarbon Solvents Panel, but also has a typical isoprene content of 1- 12%. The non-genetic endpoints can be addressed by the data for isopentane. Evaluation of the data for isopentane, along with data for Pyrolysis C5s and Hydrotreated C5s streams (containing 14-20% and 2% isoprene, respectively), can be used to characterize the genetic toxicity of this stream that contains 1 - 12% isoprene. Because the composition of this stream is substantially saturated alkanes, no additional testing is proposed for this stream.

9. 2-Methyl-2-Butene

This stream is typically 93% 2-methyl-2-butene and 6.7% 2-methyl- 1-butene. 2-Methyl-2-butene is sold as a high-purity material and is also a component in the Pyrolysis C5s, Hydrotreated C5s, Pentenes, Isoprene Concentrate, Piperylene Concentrate, and Isoprene-Piperylene Concentrate streams. The SIDS human health data set (except for the acute inhalation toxicity test which is not deemed informative for the HPV Challenge Program) will be completed for 2-methyl-2-butene. Genetic toxicity tests are completed. 2-Methyl-2-butene, like isoprene, was positive in the mouse chromosome aberration test but negative in the bacterial gene mutation test. A rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422) will be conducted. 2-Methyl-2-butene is also sponsored by the Olefins Panel in the ICCA HPV Program.

10. Metathesis Byproduct

This stream typically contains approximately 3% 1-pentene, 41% 2-pentene, 4% 1-hexene, 15% 2-hexene, 8% 3-hexene, 24% methyl-2-pentenes, and 3% 2-butene. The toxicity of the hexenes (51%) found in this stream will be evaluated by the American Chemistry Council Higher Olefins Panel's HPV program. The Higher Olefins Panel will test a C6 alkenes stream that is comprised of a mixture of branched and linear internal alkenes. Data exists for the C6 alpha olefin, 1-hexene, which was included in an OECD SIDS testing program. Based on structural similarity to the C6 alpha and internal alkenes, the pentenes found in this stream are expected to have toxicological profiles similar to those of the hexenes. In addition, 1-pentene and 2-pentene are present in the Pyrolysis C5 stream that will be tested. The toxicity of Metathesis Byproduct therefore can be characterized by data from the C6 alkenes stream, 1-hexene, and the Pyrolysis C5 stream. No additional testing of this stream is proposed at this time.

C. **Physical-Chemical Properties**

The physicochemical endpoints for the HPV Program include melting point, boiling point, vapor pressure, water solubility, and octanol water partition coefficient (K,,). Although some of these data for product streams in the C5 Non-Cyclics category exist, not all of these endpoints are defined and a

consensus database for chemicals that represent product streams in this category does not exist. Therefore, calculated physicochemical data for selected chemical components in the C5 Non-Cyclics category will be developed using the EPIWIN© computer model', as discussed in the US EPA document entitled 'The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program[2]". The use of computer modeling for the development of these data is justified since components of the streams in this category are all chemically related and are expected to exhibit minimally different environmental properties based upon the minor structural variations between these chemicals. Measured data will also be identified from the literature for selected chemical components of streams in this category. In addition, measured physicochemical data will be developed for two streams, Pyrolysis C5s and Hydrotreated C5s, which have been identified for human and environmental health testing.

Robust summaries characterizing the physicochemical endpoints will be prepared upon completion of proposed testing and will be based on available data, which will include the testing results, calculated data, and existing measured literature data. The decision to use existing measured data to characterize physicochemical endpoints will be made once the new test data are available and it is determined based on the new data that the existing data are appropriate for this purpose.

D. Ecotoxicity

Aquatic endpoints for the HPV Program include acute toxicity to a freshwater fish and invertebrate, and toxicity to a freshwater alga. The product streams of this category are expected to cause a narrow range of toxic potencies to these species regardless of the varying constituent composition of those products. This initial assessment is based on existing data for products that can be used to read across to this category and results of computer modeling using ECOSAR for selected chemical components of product streams in this category [ECOSAR is an aquatic toxicity modeling program and is a subroutine contained in EPIWIN']. The relatively narrow range of toxicity is expected because:

- Product streams in this category are composed primarily of C5 and/or C6 hydrocarbons.
- Constituent chemicals of product streams in this category are neutral organic hydrocarbons whose toxic mode of action is non-polar narcosis and whose potencies are nearly equivalent.

The mechanism of short- term toxicity for these chemicals is disruption of biological membrane function[3], and the differences between measured toxicities (i.e., LC/LL50, EC/EL50) can be explained by the differences between the target tissue-partitioning behavior of the individual chemicals[4]. The existing fish toxicity database for narcotic chemicals supports a critical body residue (CBR, the internal concentration that causes mortality) of between approximately 2- 8 mmol/kg fish (wet weight) [5,6], supporting the assessment that these chemicals have equal potencies. When normalized to lipid content, the CBR is approximately 50 umol of hydrocarbon/g of lipid for most organisms[7]. Because the product streams in this category are complex mixtures containing similar homologous chemicals, their short- term toxicities are expected to fall largely within the range of toxicity identified by the calculated data for the chemicals in Table 1.

TABLE 1: Matrix of Aquatic Toxicity Data for C_5 and C_6 Olefms and Paraffins as Calculated by the ECOSAR Computer Model.

Chemical	Fish Acute Toxicity 96-br LC50 (mg/L)	*Daphnia* Acute Toxicity 48-hr EC50 (mg/L)	Algae Toxicity 96-hr EC50 (mg/L)	Kow* Value Used for Modeling
1 -Pentene (C5)	12.5	14.0	9.1	2.66
Pentane (C5)	9.5	10.7	7.0	2.80
1 -Hexene (C6)	5.2	6.0	4.0	3.15
Hexane (C6)	3.8	4.5	3.0	3.30

* As calculated by the KOWIN subroutine in EPIWIN

Evidence to support that the range of effect values in Table 1 are expected for product streams in this category comes from an acute fish study for a C6 rich olefin product, which resulted in a 96-l-n LL50 for rainbow trout (*Oncorhynchus mykiss*) of 12.8 mg/L. Additional supportive data come from a second rainbow trout study with n-pentane. Although this is an alkane, its acute toxicity is expected to be similar to that of a C5 olefin. Comparison of a calculated value for this chemical to the measured value further supports the reliability of the calculated values for the C5 and C6 chemicals in Table 1. The ECOSAR fish 96-hour LC50 value for n-pentane is 9.5 mg/L, while the measured value is 4.3 mg/L. Determining the aquatic toxicity of products that have relatively low water solubility and higher vapor pressure, like those in this category, can be difficult because they tend not to remain in solution. These data show that the measured and calculated values are in good agreement, and they also support that the test methods used procedures that were able to maintain exposures to a reasonable extent.

To characterize the expected toxic effects in each of three aquatic organisms for product streams in this category, the Panel will test the following streams and chemicals:

- Pyrolysis C5s: A product stream containing C5 mono-olefins that are present in the streams of the C5 Non-Cyclics category, dicyclopentadiene, and significant amounts of C5 dienes.
- Hydrotreated C5s: A product stream containing very small amounts of C5 dienes; no dicyclopentadiene; and substantial concentrations of pentenes, pentanes, cyclopentene.
- 2-Methyl-2-butene (also sponsored by the Olefms Panel in the ICCA HPV Program).
- Isoprene (also sponsored by the Olefins Panel in the ICCA HPV Program).

The testing for all these substances will include an alga toxicity test (OECD Guideline 201), a *Daphnia* sp. acute toxicity test (OECD Guideline 202), and a fish acute toxicity test (OECD Guideline 203). All tests will follow testing procedure guidance for complex substances as described *in Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures*.[8]

Several streams in this category contain isoprene and 2-methyl-2-butene. With respect to the HPV

Program endpoints, these two chemicals are expected to demonstrate similar effect values, and the toxicity is expected to be equivalent to the toxicity of the streams, which are expected to be equivalently toxic. To confirm these assertions, once the testing program has been completed, the Panel will have a complete data set for isoprene, 2-methyl-2-butene and the two mixed streams. The toxicity of two streams will be compared with each other and with isoprene and 2-methyl-2-butene.

The results of the proposed testing for this category will characterize the aquatic toxicity of C5 chemicals as well as streams containing primarily C5 chemicals that also contain smaller amounts of C6 chemicals, which describes the majority of the streams in this category. Individually, C6 chemicals are expected to exhibit slightly lower effect values than the C5 chemicals. To characterize the potentially greatest toxicity of products in this category that contain primarily C6 chemicals and/or a significant proportion of C6 chemicals, the existing acute fish data for the C6 rich olefin product described above will be used. In addition, daphnid acute toxicity and alga toxicity data for a C6 olefm product will be made available to the Olefins Panel through a testing program committed to by the Higher Olefins Panel of the American Chemistry Council. The existing and planned data for the C6 olefin product will comprise a complete set of data, which in combination with results fi-om the proposed studies will provide a sufficiently robust data set to adequately characterize all the product streams in this category.

Although product streams in this category contain low levels of chemicals with carbon numbers outside the C5 to C6 range, consideration of their individual toxicities is not needed because their influence on product stream toxicity will be adequately characterized by results from the testing planned for the two streams. It must be remembered that all the chemical components of this category act by a similar mode of toxicity, nonpolar narcosis. Furthermore, it would be an inappropriate application of "read across" techniques if toxicity data for individual chemicals that comprise a relatively minor percent composition of a product were used to characterize the potential range of toxicity for products in this category. Therefore, no additional testing will be conducted to examine the influence or lack of influence fi-om minor chemical components to the range of toxicity that will be characterized by this plan. Unless the planned testing produces unexpected results, the range of toxicity for this category will be characterized by data from this testing plan and the Higher Olefins Panel testing plan.

E. Environmental Fate

Environmental fate endpoints for the HPV Program include biodegradation, photodegradation, hydrolysis, and fugacity. Biodegradation data available for two products suggest that product streams in this category have the potential to exhibit a relatively rapid rate of biodegradation. Data and/or information in the form of a technical discussion will be provided for photodegmdation. Chemicals in this category are not subject to hydrolysis at measurable rates, therefore information for this endpoint will be summarized in a technical review document. Equilibrium computer models are used to calculate chemical fugacity, which provides information on where a chemical is likely to partition in the environment. These data are useful in identifying environmental compartments that could potentially receive a released chemical. Fugacity data can only be calculated. For the HPV Program, environmental partitioning data will be calculated for selected chemical components. Preliminary data show that

chemicals in the C5 Non-Cyclics category are calculated to partition largely to the air. In addition, they have relatively low Kow values, which suggest that they will not tend to partition to suspended organic matter in air to a significant degree and precipitate to aquatic and terrestrial compartments. Because the air phase may be the primary partitioning compartment for chemicals in this category, data characterizing their potential for physical degradation in the atmosphere will be developed (this is discussed below under photodegradation).

1. Biodegradation

To investigate the biodegradability of products in this category, the Panel will test the following streams and chemicals via manometric respirometry (OECD Guideline 30 1 F):

- Pyrolysis C5s: A product stream containing C5 mono-olefins that are present in the streams of the C5 Non-Cyclics category, dicyclopentadiene, and significant amounts of C5 dienes.
- Hydrotreated C5s: A product stream containing very small amounts of C5 dienes; no dicyclopentadiene; and substantial concentrations of pentenes, pentanes, cyclopentene.
- 2-Methyl-2-butene (also sponsored in the by the Olefins Panel in the HPV Program).
- Isoprene (also sponsored by the Olefms Panel in the ICCA HPV Program).

The manometric respirometry OECD 30 1 F test guideline uses a closed test system, which is recommended when assessing the biodegradability of volatile materials like those in this category. It is also recommended when evaluating complex substances containing several chemical species, some of which may have minimally water-soluble components,

These data once developed will be evaluated against existing data to assess the biodegradability of this category. The majority of the product streams in this category are mixtures represented by the two streams in this testing plan. Results from the proposed testing, in conjunction with existing data, will adequately characterize the potential biodegradability of product streams in this category.

The existing data associated with this category are for a component chemical, isoprene. These data suggest that isoprene biodegmdes slowly. However, the data were developed using an inoculum derived from several environmental sources and incubated in the laboratory prior to use. So as to develop a comparable data set for all the substances that will be evaluated in this category, isoprene will be retested. Although individual chemical biodegradability alone may not adequately characterize the potential biodegradability of streams that are complex mixtures, it can be of interest to consider those data and evaluate other single chemicals to develop a better understanding of the fate of a complex product. The biodegradability of isoprene and 2-methyl-2-butene will be evaluated for that purpose with the intention of examining all data upon completion of the planned testing in order to adequately characterize the potential biodegradability of all product streams in this category.

2. Photodegradation -- Photolysis

Direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may lead to a chemical transformation. Simple chemical structures can be examined to determine whether a chemical has the potential for direct photolysis in water. First order reaction rates can be calculated for some chemicals that have a potential for direct photolysis using the procedures of Zepp and Cline.[9] UV light absorption of the 10 streams in the category will be evaluated to identify those chemicals having the potential to degrade in solution. For those compounds with a potential for direct photolysis in water, first order reaction rates will be calculated. If instead, a low potential for direct photolysis is suggested by the evaluation, a technical discussion will be prepared to summarize the findings.

3. Photodegradation Atmospheric Oxidation

Photodegradation can be measured" (EPA identifies OECD test guideline 113 as a test method) or estimated using models accepted by the EPA.[2] An estimation method accepted by the EPA includes the calculation of atmospheric oxidation potential (AOP). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation. AOPs can be calculated using a computer model. Light hydrocarbons, such as those in the C5 Non-Cyclics category, readily volatilize to air. In air, chemicals may undergo reaction with photosensitized oxygen in the form of ozone and hydroxyl radicals. The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows)' is used by OPPTS (Office of Pollution Prevention and Toxic Substances). This program calculates a chemical half-life based on an overall OH- reaction rate constant at a given OH- concentration. This calculation will be performed for the representative chemical components of the 10 streams in the C5 Non-Cyclics category.

4. Stability in Water (Hydrolysis Testing and Modeling)

Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters.[11] Stability in water can be measured[10] (EPA identifies OECD test guideline 111 as a test method) or estimated using models accepted by the EPA[2]. An estimation method accepted by the EPA includes a model that can calculate hydrolysis rate constants for esters, carbamates, epoxides, halomethanes, and selected alkylhalides. The computer program HYDROWIN (aqueous hydrolysis rate program for Microsoft windows)] is used by OPPTS.

All of the chemical structures included in the C5 Non-Cyclics category are simple hydrocarbons. That is, they consist entirely of carbon and hydrogen. As such they are not expected to hydrolyze at a measurable rate. A technical document will be prepared that discusses the potential hydrolysis rates of these substances, the nature of the chemical bonds present, and the potential reactivity of this class of chemicals with water.

5. Fugacity Modeling

Fugacity based multimedia modeling can provide basic information on the relative distribution of chemicals between selected environmental compartments (i.e., air, soil, sediment, suspended sediment, water, biota). The US EPA has acknowledged that computer modeling techniques are an appropriate approach to estimating chemical partitioning (fugacity is a calculated endpoint and is not measured). A widely used fugacity model is the EQC (Equilibrium Criterion) model[12]. EPA cites the use of this model in its document titled *Determining the Adequacy of Existing Data"*, that was prepared as guidance for the HPV Program.

In its document, EPA states that it accepts Level I fugacity data as an estimate of chemical distribution values. The input data required to run a Level I model include basic physicochemical parameters; distribution is calculated as percent of chemical partitioned to 6 compartments described above within a defmed unit world. Level I data are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical is likely to partition.

The EQC Level I is a steady state, equilibrium model that utilizes the input of basic chemical properties including molecular weight, melting point, vapor pressure, and water solubility to calculate distribution within a unit world. This model will be used to calculate distribution values for representative chemical components identified in streams in this category. A computer model, EPIWIN -- version 3.04[1], will be used to calculate the physicochemical properties needed to run the Level I EQC model.

Iv. **TEST PLAN SUMMARY**

The following evaluations, testing, modeling, and technical discussions will be developed for the C5 Non-Cyclics category (Table 4):

- Conduct tests for all SIDS human health endpoints (except acute toxicity) on Pyrolysis C5s, a stream typically containing approximately 14-20% isoprene, significant amounts of other dienes (e.g., cyclopentadiene, pentadienes, and dicyclopentadiene), and most of the C5 mono-olefins (including 1-5% 2-methyl-2-butene) that are present in the other streams in the C5 Non-Cyclics category (exact composition to be determined at the time of testing). The following studies will be conducted: An Ames test (OECD Guideline 47 1), a mouse inhalation micronucleus test (OECD Guideline 474), and a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422).

- Conduct tests for all SIDS human health endpoints (except acute toxicity) on Hydrotreated C5s, a stream typically containing approximately 2% isoprene; with small amounts of other C5 dienes; no dicyclopentadiene; substantial concentrations of pentenes, pentanes, and cyclopentene; and $\leq$11% 2-methyl-2-butene (exact composition to be determined at the time of testing). The following

studies will be conducted: An Ames test (OECD Guideline 471), a mouse inhalation micronucleus test (OECD Guideline 474), and a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422).

- Conduct a rat inhalation combined repeated dose/reproductive and developmental effects/neurotoxicity screen (OECD Guideline 422) on a high purity 2-methyl-2-butene stream (exact composition to be determined at the time of testing). 2-Methyl-2-butene is also sponsored by the Olefins Panel in the ICCA HPV Program.

- Evaluate all data for human health endpoints obtained from testing in this program for the Pyrolysis C5s stream, the Hydrotreated C5s stream, and 2-methyl-2-butene, along with data for components of the streams (existing data [Table 5] and data for components generated in other testing programs [see page 7]) and prepare a technical discussion in terms of their representation of potential human health effects for streams in this category.

- Calculate physicochemical data as described in the EPA document titled, *The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program.* Identify available measured physicochemical data for representative products of this category. Develop appropriate measured physicochemical data for the Pyrolysis C5s stream and Hydrotreated C5s stream.

- Conduct alga toxicity tests (OECD Guideline 201), *Daphnia* sp. acute toxicity tests (OECD Guideline 202), and fish acute toxicity tests (OECD Guideline 203) with two representative streams (Pyrolysis C5s and Hydrotreated C5s), isoprene, and 2-methyl-2-butene.

- Conduct biodegradation tests (OECD Guideline 301F) with two representative streams (Pyrolysis C5s and Hydrotreated C5s), isoprene, and 2-methyl-2-butene.

- Prepare a technical discussion on the potential of chemical components comprising streams in this category to photodegrade.

- Prepare a technical discussion on the potential of chemical components comprising streams in this category to hydrolyze.

- Calculate fugacity data for selected chemical components of streams in this category.

Summaries of results will be developed once the data and analyses are available. This test plan is expected to provide adequate data to characterize the human health effects and environmental fate and effects endpoints for the category under the Program, After all indicated testing has been completed, all data will be evaluated to determine whether the data support the category or if additional data or testing is needed.

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Table 2. CAS Numbers and Descriptions Associated with Streams in C5 Non-Cyclics Category

CAS Number	CAS Number Description
513-35-9	2-Butene, 2-methyl-
64742-83-2	Naphtha, petroleum, light steam-cracked
68410-97-9	Distillates, petroleum, light distillate hydrotreating process, low-boiling
68476-43-7	Hydrocarbons, C4-6, C5-rich
68476-55-l	Hydrocarbons, C5-rich
68477-35-O	Distillates, petroleum, C3-6, piperylene-rich
685 14-39-6	Naphtha, petroleum, light steam-cracked, isoprene-rich
68527-l 1-7	Alkenes, C5
68527-19-5	Hydrocarbons, Cl-4, debutanizer fraction
68603-00-9	Distillates, petroleum, thermal cracked naphtha and gas oil
68603-03-2	Distillates, petroleum, thermal cracked naphtha and gas oil, extractive
68606-29-l	Hydrocarbons, C4 and C8, butene concentrator by-product
68606-36-o	Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product
68956-55-8	Hydrocarbons, C5-unsatd.
78-79-5	1,3-Butadiene, 2-methyl-

Note: The definitions, found in the TSCA Chemical Substance Inventory, for the CAS numbers included in this group are vague with respect to composition. Therefore, it is not uncommon to find that the same CAS number is correctly used to describe different streams (compositions) or that two or more different CAS numbers are used to describe the same stream (composition)

Table 3. Typical Stream Compositions (%) 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
Isoprene (2-Methyl-1,3-Butadiene)	9-25		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-Butadiene	0-3							o - 1		
1,2-Butadiene					1.2					
1,3-Cyclopentadiene	2-23	5		1 - 4	o- 15	2		0-1		
Dicvclouentadiene	1 - 19			O-1	0.4					
Cyclopentene	1 - 11	15 - 20		8-20	o- 10	4				
Cyclopentane	0-2	2		1-5	8-15	1				
Isopentene		15-20								
I-Pentene (Amylene)	3-12		13.7		o- 10	6		3-11		3
2-Pentene (isomer mix)	2 - 10		18	1 - 10	1 - 16	5		3-9		41
2-Methvl-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	
3-Methyl-1-Butene (Isoamylene)	0-12				O - 1	1				
Methyl Butenes								3-21		
Isopentane (2-Methylbutane)	3-29	15-25	12.3		21	16		50-70		
n-Pentene		10 - 15								
Pentane	4-30	15-20	31.7	0-5	0 -26	16		o- 10		
3-Methvl- 1.4-Pentadiene				1.7						
3-Methyl-1-Pentene	0-2									

Component	Pyrolysis C5s	Hydrotreated C5s	Pentenes	Piperylene Concentrate	Isoprene Concentrate	Isoprene - Piperylene Concentrate	Isoprene	Isoprene Purification Byproduct	2-Methyl-2-Butene	Metathesis Byproduct
Methylpentenes				5						
C6 Hydrocarbons	2 - 4		1	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	o - 1	1		0.2						
Dimers of CPD with other C4 and C5 Dienes, excluding DCPD	o - 2									
2-Butyne (Dimethylacetylene)	o - 2				1 - 2					
1-Butene		2								
2-Butene (isomer mix)	0 - 1				1 - 20					3

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 numbers included in this group are vague with respect to composition. Therefore, it is not uncommon to find that the same CAS number is correctly used to describe different streams (compositions) or that two or more different CAS numbers are used to describe the stream (composition).

Table 4. Assessment Plan for C5 Non-Cyclics Category Under the Program. Robust summaries for existing studies are submitted separately.

Stream Description	Human Health Effects						Ecotoxicity			Environmental Fate				
	Acute Toxicity	Genetic Point Mut.	Genetic Chrom.	Sub-chronic	Develop-mental	Reprodu c-tion	Acute Fish	Acute Invert.	Algal Toxicity	Physical Chem.[1]	Photo-deg.	Hydro-lysis	Fugacity	Biodeg, I
Isoprcnc (1,3-Butadicnc, 3-methyl) , High Purity (Isoprene Content = 100%) *	√	√	√	√	√	√	T	T	T	CM	CMITD	TD	CM	T
Isoprene Concentrate (Isoprene Content = 14-85%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CM/TD	TD	CM	RA
Pyrolysis C5s (Isoprene Content = 14-20%)	NA	T	T	T	T	T	T	T	T	T	CMITD	TD	CM	T
Isoprene-Piperylene Concentrate (Isoprene Content = 20%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CMITD	TD	CM	RA
soprene Purification Byproduct (Isoprene Content = 1-12%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CMITD	TD	CM	RA
Piperylene Concentrate (Isoprene Content = 0-6%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CM/TD	TD	CM	RA
Pentenes (Isoprene Content = 2%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CMITD	TD	CM	RA
Hydrotreated C5s (Isoprene Content = 2%)	NA	T	T	T	T	T	T	T	T	T	CMITD	TD	CM	T
2-Methyl-2-Butene (≥93%)* (Isoprene Content = 0%)	NA	√	√	T	T	T	T	T	T	CM	CMITD	TD	CM	T
Metathesis Byproduct (Pentenes, Hexenes) (Isoprene Content = 0%)	RA	RA	RA	RA	RA	RA	RA	RA	RA	CM	CMITD	TD	CM	RA

1 Measured data for selected physicochemical endpoints will be identified in conjunction with calculated data to characterize this category or chemical.

√ Adequate existing data available
CM Computer Modeling proposed
NA Not Applicable
TD Technical Discussion proposed
T Testing proposed
RA Read Across (see Sec. III.B)
* Also sponsored in the ICCA Program

Table 5. Existing Data for Components Other Than Isoprene and 2-Methyl-2-Butene
(Robust summarfes for these studies will not be submitted with the Test Plan; some studies have not been reviewed for adequacy)

		Human Health Effects						Ecotoxicity			Environmental Fate				
CAS Number	**Chemical Name**	**Acute Oral**	**Genetic Point Mutation**	**Genetic Chrom. Aberr.**	**Sub-chronic**	**Develop-mental**	**Repro-duction**	**Acute Fish**	**Acute Invert.**	**Algal Toxicity**	**Physical Chem.**	**Photo-degra-dation**	**Hydro-lysis**	**Fugacity**	**Biodegra-dation**
78-78-4	Isopentane (2-methyl-butane)	√	√		√										√
590-18-1	Cis-2-butene	√													
109-66-0	Pentane	√	√	√	√	√		√	√	√					√
107-83-5	2-Methylpentane (isohexane)				√										
646-04-8	Trans-2-pentene				√										
142-29-O	Cyclopentene	√													
287-92-3	Cyclopentane	√			√										
504-60-g	1,3-Pentadiene (SIDS SIAR complete)	√	√	√	√	√	√	√	√	√					√
542-92-7	Cyclopentediene	√			√										
26760-64-5	2-methyl-1-butene			√											
77-73-6	Dicyclopentadiene	√	√	√	√	√	√	√	√	√					√
68526-52-3	Alkenes, C6 (Mix of C6 internal alkenes, 60-74% branched)		√	√				√							√
110-54-3	Hexane	√	√	√	√	√	√	√	√						

Table 6. American Chemistry Council Olefms Panel Sponsored HPV Test Categories

Category Number	Category Description
1	Crude Butadiene C4
2	Low Butadiene C4
3	C5 Non- Cvclics
4	Propylene Streams (C3) - Propylene sponsored through ICCA
5	High Benzene Naphthas
6	Low Benzene Nauhthas
7	Resin Oil - High Dicyclopentadiene
8	Resin Oil - Low Dicyclopentadiene
9	Cyclodiene Concentrates
10	Fuel Oils

Appendix I

ETHYLENE PROCESS DESCRIPTION

A. The Ethvlene 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 products. 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 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 liquid feedstocks are also converted to ethylene. While the predominant products produced are ethylene and propylene, a wide range of additional products are also formed. These products range from methane (Cl) through fuel oil (Cl2 and higher) and include other olefins, diolefins, aromatics and saturates (naphthenes and paraffms).

2. Refinery Gas Separation

Ethylene and propylene are also produced by separation of these olefins from refinery gas streams, such as from the light ends product of a catalytic cracking process or from coker offgas. 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 refmery 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 refmery gas streams yields primary ethylene and ethane, and/or propylene and propane.

B. Products of the Ethvlene Process

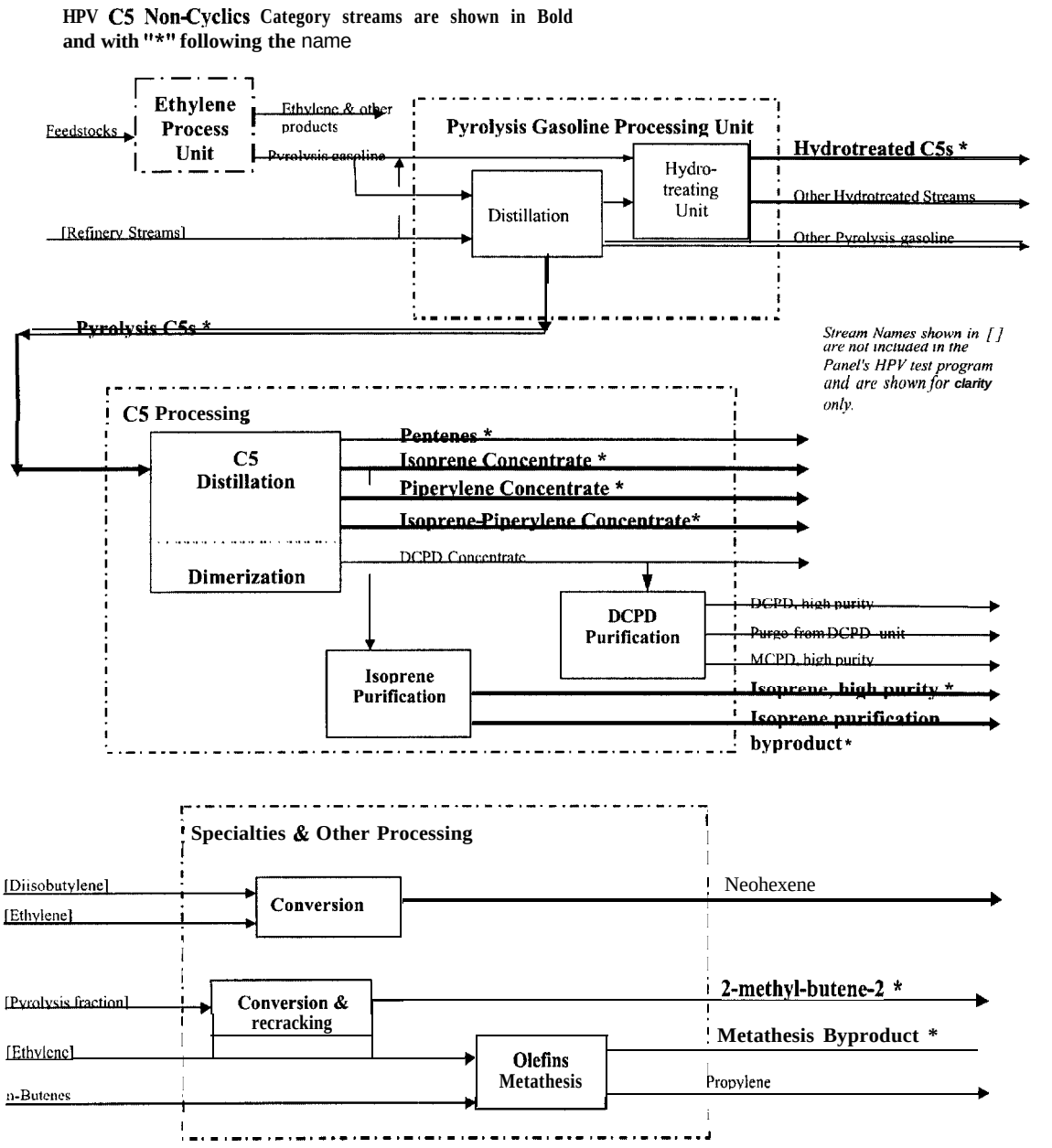
The intermediate stream that exits the cracking furnaces (i.e., the furnace effluent) is forwarded to the finishing section of the ethylene plant. The furnace effluent is commonly referred to as "cracked gas" and consists of a mixture of hydrogen, methane, and various hydrocarbon compounds with two

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 as impurities 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 pressure 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 and in some cases further processed. It is a subset of these mixed streams that make up the constituents of the C5 Non-Cyclics category.

The chemical process operations that are associated with the process streams in the C5 Noncyclics category are shown in Figure 1.

Figure 1. Chemical process operations associated with process streams in the C5 Noncyclics category.



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Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	Isoamylene, CAS# 26760-64-5
Remarks	(90% 2-Butene, 2-methyl; 10% 1 -Butene, 2-methyl).
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1990.
Species	Mouse.
Strain	$B_6C_3F_1$
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1034, 3258 or 10,350 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Remarks for Test Conditions.	Ten male $B_6C_3F_1$ mice (weighing 22-26 g, approximately 8-9 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1034, 3258, or 10,350 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced a statistically significant ($p<0.01$) and dose-related increase in micronucleated PCEs at 3258 and 10,350 ppm. The mean micronucleated PCE values were 15.7 and 31.5 at 3258 and 10,350 ppm, respectively, compared to 2.6 micronucleated PCEs for the negative control and 4.6 at 1034 ppm. The positive control produced a statistically significant increase in micronucleated PCEs (29.1). Statistically significant ($p<0.01$) and dose-related decreases in the mean percent PCEs, which is a measure of hematotoxicity, were also observed at 3258 and 10,350 ppm. The %PCEs were 58.7, 59.6, 54.4, and 40.5% at 0, 1034, 3258, and 10,350 ppm. The %PCEs for the positive control was 42.0%.
Conclusions (study author)	Under the conditions of this study, inhalation exposure to 3258 and 10,350 ppm of the test substance induced statistically significant increases in micronucleated polychromatic erythrocytes in male $B_6C_3F_1$ mice.
Data Quality	
Reliability	1 - Reliable without restrictions.
Reference<	ExxonMobil Biomedical Sciences, Inc. (1990). In Vivo Mammalian Bone Marrow Micronucleus Assay. Unpublished study.
Other	
Last changed	16-May-01 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vitro

Test Substance	2-Butene, 2-methyl. CAS# 5 13-35-9
Remarks	(2-methyl-2-butene, 85% purity)
Method	
Method/guideline followed	OECD 47 1
Type	Ames Salmonella/bacterial reverse mutation test (pre-incubation assay).
System of testing	Bacterial.
GLP	Yes.
Year	*1980.*
Species/Strain	*Salmonella typhimurium/* TA98, TA100, TA1535, TA1537, TA1538
Metabolic activation	With and without.
Species and cell type	Rat liver S9 fraction.
Quantity	0.5 ml/plate.
Induced or not induced	Arochlor 1254-induced.
Concentrations tested	0, 0.2, 2, 20, 500, and 2000 ug/plate.
Statistical Methods	A positive response was defined as a minimum consistent doubling of the spontaneous reversion frequency, or if the number of induced revertants is less than twice the spontaneous rate then a reproducible, dossrelated increase in any one strain/activation combination was interpreted as positive.
Remarks for Test Conditions	The preincubation modification of the *Salmonella*/mammalian microsome assay was tested in five different *Salmonella* strains in the presence and absence of rat liver S-9. Five dose levels were tested, with three plates per dose level. Bacteria (0.5 ml) and S9 mix or pH 7.4 phosphate buffer (2.5 ml) were incubated at 37°C with the test substance in ethanol (0.1 ml) 30 minutes before incorporation of 0.5 ml of this mixture into 2 ml of top agar. Concurrent positive and solvent controls were also tested with and without metabolic activation. Two replicate assays were performed on different days to confirm the reproducibility of the results.
Results	
Genotoxic effects	Negative. The test substance was not mutagenic in any of the five strains of Salmonella tested in the presence or absence of metabolic activation (rat liver S9).
Conclusions (contractor)	The test substance was not mutagenic in the Ames Salmonella mutagenicity test.
Data Quality *Reliabilities*	1 - Reliable without restrictions.
Reference	Dean, B.J., Brooks, T.M., Hodson-Walker, G., and Hutson, D.H. (1985). Genetic toxicology testing of 41 industrial chemicals. Mutation Research 153:57-77.
Other	
Last changed	16-Oct-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Nou-Cyclics

Genetic Toxicity - in Vivo

Test Substance	2-Butene, 2-methyl. CAS# 513-35-9
Remarks	2-methyl-2-butene, >99.2% purity)
Method	
Method/guideline followed	DECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes
Year	1991.
Species	Mouse.
Strain	$B_6C_3F_1$
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1005, 3207, or 9956 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control), IO males exposed to 1000 ppm 1,3 butadiene (positive control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Remarks for Test Conditions.	Ten male $B_6C_3F_1$ mice (weighing 24-28 g, approximately 6-7 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1005, 3207 or 9956 ppm (analytical mean) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced statistically significant ($p<0.01$) and dose-related increases in micronucleated PCEs at 3207 and 9956 ppm. The mean micronucleated PCE values were 4.2, 16.6 and 36.1 at 1005, 3207 and 9956 ppm, compared to 3.4 micronucleated PCEs for the negative control. The positive control produced a statistically significant increase in micronucleated PCEs (29.7). A statistically significant ($p<0.01$) decrease in the %PCEs, which is a measure of hematotoxicity, was also observed at 9956 ppm. The %PCEs were 57.4, 57.4, 54.3, and 37.9% at 0, 1000, 3207, and 9956 ppm. The %PCEs for the positive control was 44.5%.
Conclusions (study author)	Under the conditions of this study, inhalation exposure to 3207 and 9956 ppm of the test substance induced statistically significant increases in micronucleated polychromatic erythrocytes in male $B_6C_3F_1$ mice.
Data Quality *Reliabilities*	1 - Reliable without restrictions
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.
Other Last changed	16-May-01 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	2-Butene, 2-methyl. CAS# 513-35-9
Remarks	(2-methyl-2-butene, >99.2% purity)
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
Species	Rat.
Strain	CrlCDBR
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	3, 1005, 3207, or 9956 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Remarks for Test Conditions.	Ten male CrlCDBR rats (weighing 295-345 g, approximately 9 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1005, 3207 or 9956 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced statistically significant ($p<0.01$) and dose-related increases in micronucleated PCEs at 3207 and 9956 ppm. The mean micronucleated PCE values were 4.2 and 4.9 at 3207 and 9956 ppm, respectively, compared to 2.7 for the negative control (air) and 2.2 at 1005 ppm. Statistically significant decreases in the mean percent PCEs, which is indicative of hematotoxicity, were also observed at all three exposure levels. Although the mean PCE frequencies at 1005, 3207 and 9956 ppm (48.6, 51.0, 49.8%, respectively) were slightly decreased form the negative control (54.9%), they were not different from each other and did not show evidence of a dose-response. Therefore, the biological significance of this observation is unclear.
Conclusions (study author)	Under the conditions of this study, inhalation exposure to 3207 and 9956 ppm of the test substance induced small but statistically significant increases in micronucleated polychromatic erythrocytes in male rats.
Data Quality Reliability	2 - Reliable with restrictions. No concurrent positive control was used.
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.
Other	
Last changed	30-Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	1-Butene, 2-methyl CAS# 26760-64-5
Remarks	(2-methyl-1-butene, >99.2% purity)
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
Species	Mouse.
Strain	$B_6C_3F_1$.
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1038, 33 12, or 10,116 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/ exposure level.
Control groups and treatment	10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Remarks for Test Conditions.	Ten male $B_6C_3F_1$ mice (weighing 2430 g, approximately 7-8 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1038, 33 12 or 10,116 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	A dose-related increase in mean micronucleated PCEs was observed (2.4, 3.7, 3.6, and 4.6 at 0, 1038, 33 12 and 10,1 16 ppm). However, since none of the exposed groups were statistically different from the negative control this finding was not considered to be biologically significant. The mean micronucleated PCE value of 4.6 at 10,1 16 ppm was slightly outside the normal range of the negative control (0-4), although it was not statistically significant ($p<0.09$). The positive control produced a statistically significant increase in micronucleated PCEs (43.1). The mean percent of PCEs were within the normal range for all exposure groups. The %PCEs were 58.5, 60.7, 59.2, and 58.8% at 0, 1038, 3312 and 10,116 ppm. The %PCEs for the positive control was 41.6%.
Conclusions (study author)	Under the conditions of this study, inhalation exposure to the test substance did not induce a statistically significant increase in micronucleated polychromatic erythrocytes in male $B_6C_3F_1$ mice.
Data Quality	
Reliability	1 - Reliable without restrictions.
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.
Other	
Last changed	16-May-01
	Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	I-Butene, 2-methyl CAS# 26760-64-5
Remarks	2-methyl-1-butene, >99.2% purity)
Method	
Method/guideline followed	3ECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
Species	Rat.
Strain	CrlCDBR
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	3, 1038, 33 12, or 10,116 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Remarks for Test Conditions.	Ten male CrlCDBR rats (weighing 337-414 g, approximately 10-l 1 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1038, 3312 or 10,116 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance did not induce a statistically significant increase in micronucleated PCEs at 24 hours in any of the exposure groups. The micronucleated PCEs were 2.0, 1.8, 1.9, and 2.7 at 0, 1038, 3312 and 10,116 ppm The mean percent PCEs were within the normal range of the negative controls. The %PCEs were 48.7, 51.7, 49.9, and 53.4% at 0, 1038, 3312 and 10,116 ppm.
Conclusions (study author)	Under the conditions of this study, inhalation exposure to the test substance did not induce a statistically significant increase in micronucleated polychromatic erythrocytes in male CrlCDBR rats.
Data Ouaiitv	
Reliability	2 - Reliable with restrictions. No concurrent positive control was used.
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay. Unpublished study.
Other	
Last changed	16-May-01 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	Isoamylene, CAS# 26760-64-5
Remarks	(-92% 2-Butene, 2-methyl; -7% 1 -Butene, 2-methyl).
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
Species	Mouse.
Strain	$B_6C_3F_1$
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1034, 3266 or 10,097 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Remarks for Test Conditions.	Ten male $B_6C_3F_1$ mice (weighing 24-30 g, approximately 8-9 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1034, 3266, or 10,097 ppm (analytical means) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced statistically significant ($p<0.01$) and dossrelated increases in micronucleated PCEs at 3266 and 10,097 ppm. The mean micronucleated PCE values were 3.7, 22.6 and 42.1 at 1034, 3266 and 10,097 ppm, compared to 2.5 micronucleated PCEs for the negative control. The positive control produced a statistically significant increase in micronucleated PCEs (39.5). Statistically significant ($p<0.01$) decreases in the mean percent PCEs, which is a measure of hematotoxicity, were also observed at 3266 and 10,097 ppm. The %PCEs were 58.2, 58.0, 5 1.4, and 34.6% at 0, 1034, 3266 and 10,097 ppm. The %PCEs for the positive control was 43.7%.
Conclusions (study author)	Under the conditions of this study, inhalation exposure to 3266 and 10,097 ppm ofthe test substance induced statistically significant increases in micronucleated polychromatic erythrocytes in male $B_6C_3F_1$ mice.
Data Quality	
Reliability	1 - Reliable without restrictions
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay. Unpublished study.
Other	
Last changed	17-May-01
	Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	
Remarks	Isoamylene, CAS# 26760-64-5 (-92% 2-Butene, 2-methyl; -7% 1 -Butene, 2-methyl).
Method	
Method/guideline followed	OECD 474.
Type	Mammalian erythrocyte micronucleus test.
GLP	Yes.
Year	1991.
species	Rat.
strain	CrlCDBR
Sex	Males.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0, 1034, 3266 or 10,097 ppm (analytical mean concentrations).
Exposure period	6 hours/day for 2 consecutive days.
No. of animals per dose	10 males/exposure level.
Control groups and treatment	10 males exposed to air (negative control).
Statistical methods	Means and standard deviations of micronuclei data. ANOVA to test for equality of group means followed by Duncan's Multiple Range Test if appropriate. Standard regression analysis to test for dose-related response. Wilk's Criterion for normality.
Remarks for Test Conditions.	Ten male CrlCDBR rats (weighing 348-447 g, approximately 1 1- 12 weeks old) per group were exposed for 2 consecutive days, 6 h/day to 0, 1034, 3266, or 10,097 ppm (actual mean exposures) of the test substance by inhalation. Exposure concentrations were determined by on-line gas chromatography. Bone marrow smears were prepared and stained 24 hours after the last exposure. 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronucleated PCEs. The proportion of PCEs to normochromatic erythrocytes (NCEs) was determined by counting a total of 1000 erythrocytes per animal.
Results	The test substance induced a statistically significant ($p<0.01$) increase in micronucleated PCEs at 10,097 ppm. The mean micronucleated PCE values were 3.4, 4.2, and 7.0 at 1034, 3266 and 10,097 ppm, compared to 3.3 micronucleated PCEs for the negative control. The slight increase in mean micronucleated PCEs (4.2) noted at 3266 ppm was slightly above the normal range for the negative control (O-4) although it was not statistically significant. The mean percent PCEs were within the normal range of the negative control for all exposed groups. The %PCEs were 48.3, 46.9, 46.1, and 45.3% at 0, 1034, 3266 and 10,097 ppm. .
Conclusions (study author)	Under the conditions of this study, inhalation exposure to 10,097 ppm of the test substance induced a statistically significant increase in micronucleated polychromatic erythrocytes in male rats.
Data Quality	
Reliability	2 - Reliable with restrictions. No concurrent positive control was used.
References	ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.
Other	
Last changed	17-May-01 Robust summary prepared by a contractor to the Panel.

AR201-13186B

Robust Summary - Group 3: C5 Non-Cyclics

Biodegradation

Test Substance	Isoprene; CAS# 78-79-5; Purity unknown
Method	
Method/guideline followed	OECD 301C, Ready Biodegradability: Modified MITI Test
Year (guideline)	Unknown
Type (test type)	Aerobic
GLP	Unknown
Year (study performed)	Unknown
Inoculum	Mixture from several sources in Japan that included 4 sewage plants, 3 rivers, 2 bays, and 1 lake.
Exposure Period	28 days
Test Conditions Note: Concentration prep., vessel type, replication, test conditions.	Inoculum: A mixed inoculum was developed and maintained that used several sources and included: return sludge from 1 industrial and 3 city sewage plants; and water from 3 rivers, 2 bays, and 1 lake, with soil from land adjacent to these bodies of water. A filtrate from the combination of these samples was prepared and added to an existing culture that had been developed from the same sources as above and maintained under aeration and with a synthetic feed composed of glucose, peptone, and monopotassium phosphate. The inoculum used for this biodegradation test was removed from the mixed culture and added to the test systems at a concentration of 2 mg of inoculum per liter of test medium. Controls: Blank and positive controls were used per guideline. Positive control was aniline added to the control vessel at a loading of 100 mg/L. Test material: Test systems contained 2 and 10 mg test substance per liter of medium. Temperature of incubation: 24 - 26°C Analytical method: Oxygen consumption was monitored using an 02 probe from Ohkura Electric Co., Ltd. Method of calculating biodegradation values: Percent biodegradation was calculated as a percent ratio of the biological oxygen demand (BOD) in the test system less the BOD of the blank control, to the calculated theoretical oxygen demand of the added test material. Test validity: The positive control percent biodegradation had to have achieved 40% and 60% by days 7 and 14, respectively, for the test to be considered valid.
Results	
Units/Value:	2% biodegradation after 28 days

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Conclusions	The validity criteria required to be met by the positive control were achieved, therefore. the test was considered valid. It is unknown how the inoculum preparation procedure affected the final population of organisms in the culture added to the test systems. Because there was no information on the robustness of the culture at test initiation, these data should be characterized as having been developed using an inoculum from several sources that was maintained as a lab culture with aeration and synthetic feed prior to use.
Data Quality *Reliabilities*	(2) Reliable with restrictions
Reference	Chemicals Inspection and Testing Institute, Japan. 1992. Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. Japan Chemical Industry Ecology-Toxicology and Information Center. ISBN 4-89074-101-1.
Other *Last* ***changed***	07-Sep-01 Robust summary prepared by a contractor to the Panel

Robust Summary - Group 3: C5 Non-Cyclics

Acute Toxicity

Test Substance	Isoprene, CAS# 78-79-S
Method	
Method/guideline followed	Other.
Type (test type)	Acute inhalation -LC_{50}
GLP	Pre-GLP
Year	1969
Species/Strain	Rat and mouse (strains not specified)
Sex	Not specified
No. of animals per sex per dose	Not specified
vehicle	Not applicable
Route of administration	Inhalation (vapor)
Test Conditions	Age, number, and sex of test animals not specified. Number of groups and exposure concentrations not specified. Dynamic flow exposure system; no description of exposure chambers or conditions. Rats exposed four hours; mice exposed two hours. No post-exposure observation period - mortality study only. Exposure concentrations "controlled" by gas chromatography. LC50 calculation by probit-analysis according to Litchfield and Wilcoxon.
Results	
LC_{50} with confidence limits.	Rat LC_{50} (4 hr) = 180 mg/L (64,620 ppm); confidence limits 130-181 mg/L ($p \leq 0.05$). Mouse LC_{50} (2 hr) = 157 mg/L (56,363 ppm); confidence limits 129-252 mg/L ($p \leq 0.05$).
Remarks	***No*** clinical observations or necropsy findings reported. Objective of study was to determine hydrocarbon concentrations in various tissues at lethal exposure concentrations.
Conclusions (study author)	LC50 value reported to be 180 mg/L (64,620 ppm) in rats, 1.57 mg/L (56,363 ppm) in mice.
Data Quality	
Reliability	Not assignable. Lethality study only; insufficient experimental detail to assess quality.
References	Shugaev, B.B. (1969) Concentrations of Hydrocarbons in Tissues as a Measure of Toxicity. Arch. Environ. Health 18:878-882.
Other	
Last changed	21-Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vitro

Test Substance	soprene, CAS# 78-79-5
Test substance	Purity >99%.
Method	
Method/guideline followed	3ECD 47 1
Type	Ames Salmonella/bacterial reverse mutation test (pre-incubation assay).
System of testing	Bacterial.
GLP	Yes.
Year	*1986*
Species/Strain	*Salmonella* / TA98, TA100, TA1535, TA1537.
Metabolic activation	With and without.
Species and cell type	Rat and hamster liver S9 fraction.
Quantity	3.5 ml/plate.
Induced or not induced	4rochlor 1254-induced (500 mg/kg for 5 days).
Concentrations tested	3, 100, 333, 1000, 3333, 10000 ug/plate.
Statistical Methods	A positive response was defined as a reproducible, dose-related increase in revertant colonies in any one strain/activation combination. There was no minimum percentage or fold increase required for the chemical to be judged positive or weakly positive.
Remarks for Test Conditions	The preincubation modification of the Salmonella/mammalian microsome assay was used to test isoprene in five different *Salmonella* strains in the presence and absence of rat and hamster liver S-9. Five dose levels were tested, with three plates per dose level. The high dose was limited by toxicity to 10,000 ug/plate. 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.
Results	
Genotoxic effects	Negative. Isoprene was not mutagenic in any of the five strains of *Salmonella* tested in the presence or absence of Aroclor-induced rat or hamster liver S9.
Conclusions (contractor)	Isoprene was not mutagenic in the Ames Salmonella mutagenicity test.
Data Quality *Reliabilities*	Reliable without restrictions. Evaluated as part of a NTP-sponsored interlaboratory study of 270 chemicals.
Reference	Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986) Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ. Mutagen. 8 (Suppl. 7): 1-1 19.
Other	
Last changed	20-Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vitro

Test Substance	soprene, CAS# 78 -79- 5
Test substance	Purity >99%.
Method	
Method/guideline followed	OECD 479
Type	*In vitro* Sister Chromatid Exchange (SCE) Assay in Mammalian Cells
System of testing	Chinese hamster ovary (CHO) cells.
GLP	Yes.
Year	1987.
Metabolic activation	4roclor 1254-induced Sprague-Dawley rat liver S9.
Concentrations tested	50, 160, 500, 1600 ug/ml (without S9), or 160,500, 1600, 5000 ug/ml (with S9).
Control groups and treatment	Solvent controls: dimethylsulfoxide; positive controls : Mitomycin-C (without S9), :yclophosphamide (with S9).
Statistical Methods	Statistical analyses were conducted on the slopes of the dose-response curves and the Individual dose points. A frequency 20% above the solvent control group was considered positive. Positive trend tests ($p \leq 0.05$) in the absence of a significant difference at any one dose were considered equivocal.
Remarks for Test Conditions	lsoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) both in the presence and absence of Amclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and four doses of isoprene. 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 2"“-division metaphase cells were scored for frequency of SCEs/cell from each dose level.
Results	
Genotoxic effects	Negative. No increases in SCEs were noted in cultured CHO cells treated with isoprene, with or without S9.
Conclusions (contractor)	lsoprene did not induce sister chromatid exchanges *in vitro* in cultures of Chinese hamster ovary cells.
Data Quality *Reliabilities*	Reliable without restrictions. Evaluated as part of a NTP-sponsored study of 108 chemicals.
Reference	Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations *of* 108 chemicals. Environ Mol. Mutagen 10:1 - 175.
Other	
Last changed	21 -Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Isoprene, CAS# 78-79-5 Purity >99%.
Method	
Method/guideline followed	OECD 473
Type	*In vitro* Mammalian Chromosomal Aberration Test.
System of testing	Chinese hamster ovary (CHO) cells.
GLP	Yes.
Year	1987.
Metabolic activation	Aroclor 1254-induced Sprague-Dawley rat liver S9.
Concentrations tested	1600, 3000, 5000 ug/ml.
Control groups and treatment	Solvent control: dimethylsulfoxide; positive controls : Mitomycin-C (without S9), cyclophosphamide (with S9).
Statistical Methods	Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points, A statistically significant ($p \leq 0.05$) difference for one point and a significant trend ($p \leq 0.015$) was considered positive. Positive trend tests ($p \leq 0.05$) in the absence of a significant difference at any one dose were considered equivocal.
Remarks for Test Conditions	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 and positive controls and three doses of isoprene. 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.
Results	
Genotoxic effects	Negative. No increases in chromosomal aberrations were noted in cultured CHO cells treated with isoprene, with or without S9.
Conclusions	
(contractor)	Isoprene did not induce chromosomal aberrations *in vitro* in cultures of Chinese hamster ovary cells.
Quality	
Reliabilities	Reliable without restrictions, Evaluated as part of a NTP-sponsored study of 108 chemicals.
Reference	Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environ Mol. Mutagen 10:1-175.
Other	
Lust changed	21 -Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >98%.
Method	
Method/guideline followed	Other.
Type	In *vivo* Sister Chromatid Exchange (mouse bone marrow cytogenetics study) .
GLP	Yes.
Year	1988.
Species	Mouse
Strain	B6C3F1.
Sex	15 male/group.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0,438, 1750, 7000 ppm.
Exposure period	6 hours/day for 12 days.
Statistical methods	The frequencies of sister chromatid exchanges (SCEs) were analyzed for increasing trend by the one-tailed Cochran-Armitrage trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using either the one-tailed or two-tailed t-test
Remarks for Test Conditions.	Fifteen male B6C3F1 mice (approximately 6-7 weeks old) per group were exposed for 12 days, 6 h/day to 0,438, 1750, or 7000 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 before sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of SCE, 5 mice per exposure group were killed 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.
Results	
Genotoxic effects	Positive.
NOAEL (NOEL)	<438 ppm.
LOAEL (LOEL)	438 ppm.
	Exposure to isoprene for 6 h/day at 0,438, 1750, or 7000 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 1750 ppm, and 13.98 at 7000 ppm). The increased SCE responses in the exposed groups were not statistically different from each other. Exposure to isoprene resulted in a statistically significant lengthening of the bone marrow Average Generation Time (AGT) at 7000 ppm (AGT = 11.68, 12.98, 12.73, and 13.72 h ar 0,438, 1750, and 7000 ppm, respectively) but did not significantly alter the mitotic index. There were no significant clinical signs or mortality throughout the study.
Conclusions (study authors)	Isoprene was found to be genotoxic and cytotoxic to mouse bone marrow in vivo - inducing SCE and causing a delay in cell cycle time. 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.
Data Quality *Reliabilities*	Reliable without restrictions. NTP-sponsored study.
References	Tice, R.R., Boucher, R., Luke, C.A., Paquette, D.E., Melnick, R.L., and Shelby, M.D. (1988). Chloroprene and isoprene: cytogenetic studies in mice. Mutagenesis 3(2):141-146.
Other *Last changed*	07-Sep-01 Robust summary prepared by a contractor to the Panel.

Genetic Toxicity - in Vivo

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >98%.
Method	
Method/guideline followed	OECD 475
Type	Mammalian Bone Marrow Chromosomal Aberration Test.
GLP	Yes.
Year	1988.
Species	Mouse
Strain	B6C3F1
sex	15 male/group.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0,438, 1750, 7000 ppm.
Exposure period	6 hours/day for 12 days.
Statistical methods	The frequencies of chromosomal aberrations (Abs) were analyzed for increasing trend by the one-tailed Cochran-Armitrage trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using either the one-tailed or two-tailed t-test
Remarks for Test Conditions.	Fifteen male B6C3F1 mice (approximately 6-7 weeks old) per group were exposed for 12 days, 6 h/day to 0,438, 1750, or 7000 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 before sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of Abs, 10 mice per exposure group were killed 17-20 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 Abs from 8 mice/group. Additionally, 100 randomly selected metaphase cells per slide were scored forreplication history to provide data on cell generation time, a measure of cell proliferation kinetics. The percentage of cells in metaphase among 1000 cells/sample was used to calculate the mitotic index.
Results	
Genotoxic effects	Negative.
NOAEL (NOEL)	7000 ppm
LOAEL (LOEL)	>7000 ppm
	Exposure to isoprene for 6 h/day at 0,438, 1750, or 7000 ppm for 12 days did not induce a statistically significant increase in the frequency of chromosomal aberrations (Abs) in bone marrow cells. The incidence of bone marrow cells with chromosomal aberrations (Abs) was slightly elevated in the exposed groups compared to the control (0.02 at 0 ppm vs. 0.04, 0.05, and 0.04 at 438, 1750, and 7000 ppm), 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 7000 ppm group was slightly increased compared to the contols (1.15% vs 1.30%). Analysis of average generation time showed a statistically significant lengthening of the cell cycle duration of proliferating cells in the 7000 ppm group (13.72 hours at 7000 ppm vs. 11.68 hours at 0 ppm).
Conclusions (study authors)	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 the controls, but were not statistically increased.
Data Quality *Reliabilities*	Reliable without restrictions. NTP-sponsored study.
References	Tice, R.R., Boucher, R., Luke, C.A., Paquette, D.E., Melnick, R.L., and Shelby, M.D. (1988). Chloroprene and isoprene: cytogenetic studies in mice. Mutagenesis 3(2):141-146.
Other	16-May-01
Last changed	Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >98%.
Method	
Method/guideline followed	OECD 474
Type	Mammalian Erythrocyte Micronucleus Test
GLP	Yes.
Year	1988.
Species	Mouse
Strain	B6C3F1
Sex	15 male/group.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0,438, 1750, 7000 ppm.
Exposure period	6 hours/day for 12 days.
Statistical methods	The number of micronucleated erythrocytes (MN) were summed across animals within each group and analyzed for increasing trend by a one-tailed trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using a one-tailed Pearson Chi square test to determine the minimal effective dose.
Remarks for Test Conditions.	Approximately 24 hours following the last exposure peripheral blood samples were obtained from each animal by tail snip, immediately air-dried and fixed with methanol. One thousand polychromatic erythrocytes (PCEs) and 1000 normochromatic erythrocytes (NCEs) were scored per animal for frequency of micronucleated erythrocytes (MN). The percentage of PCEs in 1000 erythrocytes was also determined as a measure of isoprene-induced toxicity.
Results	
Genotoxic effects	Positive.
NOAEL (NOEL)	<438 ppm.
LOAEL (LOEL)	438 ppm.
	Exposure to isoprene for 6 h/day at 0,438, 1750, or 7000 ppm for 12 days induced a statistically significant increase in the frequency of MN-PCEs and NCEs in male mice at all exposure levels tested. The frequencies of MN-PCEs were 2.00, 12.00, 15.60, and 16.93 at 0, 438, 1750, and 7000 ppm. The responses at the 1750 and 7000 ppm levels both were greater than the 438 ppm level, but not statistically different from each other. There also was a dose-related decrease in the percentage of PCEs, a measure of the rate erythropoiesis (3.91, 3.00, 2.87, and 1.64 at 0,438, 1750, and 7000 ppm). There were no significant clinical signs or mortality throughout the study.
Conclusions	
(study authors)	Isoprene was found to be genotoxic to mouse bone marrow *in vivo* by inducing increased MN in the peripheral blood of male mice. Suppression of erythropoiesis was suggested by decreased percentage of PCEs.
Data Quality	
Reliabilities	Reliable without restrictions. NTP-sponsored study.
References	Tice, R.R., Boucher, R., Luke, C.A., Paquette, D.E., Melnick, R.L., and Shelby, M.D. (1988). Chloroprene and isoprene: cytogenetic studies in mice. Mutagenesis 3(2):141-146.
Other	
Last changed	07-Sep-0 1 Robust summary prepared by a contractor to the Panel

Robust Summary - Group 3: C5 Non-Cyclics

Genetic Toxicity - in Vivo

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99.7%.
Method	
Method/guideline followed	Other.
Type	Rat Lung Fibroblast Micronucleus Test
GLP	Yes.
Year	1997.
Species	Rat
Strain	Fischer 344
Sex	10 male and 10 female/group.
Route of administration	Inhalation (vapor).
Doses/concentration levels	0,220, 700, or 7000 ppm.
Exposure period	6 hours/day, 5 days/week, for 4 weeks.
Statistical methods	Means, standard deviations, and standard error of the mean for the number of mononucleated cells/1000 binucleated cells and micronuclei/1000 binucleated cells were calculated. A two-way analysis of variance was used to analyze the measurements. Intergroup differences were delineated by Tukey's studentized range test.
Remarks for Test Conditions.	This study was performed in conjunction with a two-year carcinogenicity study. Groups of 10 male and 10 female rats (approximately 6-7 weeks old) per group were exposed for 4 weeks (17- 19 total exposures) to 0, 220, 700, or 7000 ppm of isoprene by inhalation. 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 (acridine orange), and 1000 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.
Results	
Genotoxic effects	Negative. 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.
Conclusions (study authors)	No significant increase in the frequency of micronucleated lung fibroblasts was observed in male and female rats exposed to isoprene for 4 weeks.
Data Quality *Reliabilities*	Reliable with restrictions. Non-standard method, but comparable to guideline study. Conducted as part of NTP two-year carcinogenicity study.
References	National Toxicology Program (1997). Toxicology and Carcinogenesis Studies of lsoprene (CAS No. 78-79-5) in F344/N Rats (Inhalation Studies). Report No. TR-486.
Other	
Last *changed*	21-Aug-00 Robust summary prepared by a contractor to the Panel.

Repeated Dose Toxicity	
Test Substance	
Remarks	Isoprene, CAS# 78-79-S Purity >99%.
Method	
Method/guideline followed	Other.
Test type	Z-week inhalation study.
GLP	Yes.
Year	1990.
Species	Rat and mouse.
Strain	F344 rats and B6C3F 1 mice.
Route of administration	Inhalation (vapor).
Duration of test	2 weeks.
Doses/concentration levels	0, 438, 875, 1750, 3500, or 7000 ppm.
Sex	20 male, 20 female per group.
Exposure period	6 hours/day.
Frequency of treatment	5 days/week.
Control group and treatment	20 male, 20 female, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	Group mean body weights, organ weights, organ weight ratios, and clinical pathology results compared to controls by Dunnett's t-test.
Test Conditions	Groups of 20 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for two weeks (10 exposures). Ten animals/sex/group/species were used for clinical pathology evaluations after 4 (rats) or 5 (mice) exposures. The remaining ten 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 (7000 ppm), and lower dose groups until an apparent no-observed -effect level was found.
Results	
NOAEL (NOEL)	7000 ppm rats, not determined for mice.
LOAEL (LOEL)	>7000 ppm rats, 438 ppm mice.
Remarks	In rats, there were no exposure-related effects observed for survival, body weight gain, clinical signs, hematologic or clinical chemistry parameters, organ weights, or the incidence of gross or microscopic lesions. In mice, there were no effects on survival; the mean body weight gain of males in the 7,000 ppm group was less than that of the controls. In mice, exposure to isoprene caused decreases in hematocrit values. hemoglobin concentrations, and erythrocyte counts in all exposed groups. Organ weight changes were observed in both male and female mice; increased liver weights and decreased thymus, spleen, and testis weights were observed in all exposed groups. Microscopic lesions observed in the exposed mice included atrophy of the testis and thymus, cytoplasmic vacuolization of the liver, olfactory epithelial degeneration in the nasal cavity, and epithelial hyperplasia in the forestomach.
Conclusions (contractor)	Isoprene exposures over 2 weeks induced changes in hematological parameters, body and organ weights, and microscopic appearances in certain tissues at levels as low as 438 ppm in the mouse whereas no changes were noted in measured parameters in the rat at exposures up to 7000 ppm. The lack of any observable toxicological effects in F344 rats exposed to isoprene for two weeks provides evidence for a species difference between rats and mice in susceptibility to isoprene.
Data Quality	
Reliabilities	Reliable without restrictions. Comparable to guideline study (OECD 412).
References	Melnick, R.L., Roycroft, J.H., Chou, B.J., Ragan, H.A., and Miller, R.A. (1990). Inhalation toxicology of isoprene in F344 and B6C3F1 mice following two-week exposures. Environ. Health Perspect. 86:93-98.
Other	
Last changed	21 -Aug-00 Robust summary prepared by a contractor to the Panel.

Repeated Dose Toxicity

Test ***Substance***	lsoprene, CAS# 78-79-5
Remarks	Purity >99%.
Method	
Method/guideline followed	Other.
Test type	13-week inhalation study.
GLP	Yes.
Year	1994.
Species	Rat and mouse.
Strain	F344 rats and B6C3F1 mice.
Route of administration	Inhalation (vapor).
Duration of test	13 weeks.
Doses/concentration levels	0, 70,220, 700, 2200, or 7000 ppm.
Sex	10 male, 10 female per group.
Exposure period	6 hours/day.
Frequency of treatment	5 dayslweek.
Control group and treatment	10 male, 10 female, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.
Test Conditions	Groups of 10 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen 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 recorded.
Results	
NOAEL (NOEL)	7000 ppm rats, 220 ppm mice.
LOAEL (LOEL)	>7000 ppm rats, 700 ppm mice.
Remarks	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. 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. The incidences of focal epithelial hyperplasia of the forestomach were 0, 0, 0, 9, 8, 9 in the males, and 0, 0, 0, 10, 9, 10 in the females at 0, 70, 220, 700, 2200, and 7000 ppm (n=10). Degeneration of the olfactory epithelium and cytoplasmic degeneration of the liver were observed in 10110 male mice at 7000 ppm. The male mice exposed to 7000 ppm exhibited testicular weights reduced 35% compared to the controls.
Conclusions (contractor)	No toxicological effects were evident in rats exposed up to 7000 ppm isoprene for 13 weeks. In mice, hematological and histopathological changes were observed at exposures of 700 ppm and higher. This 13-week subchronic inhalation study, conducted as part of a 26-week carcinogenicity study, confirmed the species difference between rats and mice in susceptibility to isoprene.
Data Quality	
Reliabilities	Reliable without restrictions. Comparable to guideline study (OECD 413).
References	Melnick RL; Sills RC; Roycroft JH; Chou BJ; Ragan HA; Miller RA (1994). Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Res. 54:5333-5339.
Other	
Last changed	21 -Aug-00 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Repeated Dose Toxicity

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99%.
Method	
Method/guideline followed	Other.
Test type	26-week inhalation study.
GLP	Yes.
Year	1994.
Species	Rat and mouse.
Strain	F344 rats and B6C3F 1 mice.
Route of administration	Inhalation (vapor).
Duration of test	26 weeks.
Doses/concentration levels	0, 70, 220, 700, 2200, or 7000 ppm.
Sex	40 male rats and 40 male mice per group.
Exposure period	6 hours/day.
Frequency of treatment	5 daysiweek.
Control group and treatment	40 male rats and 40 male mice, air-only exposed.
Post exposure observation period	26-week post-exposure recovery period.
Statistical methods	Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.
Test Conditions	Groups of 40 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 26 weeks. 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 recovery 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.
Results t s)	
NOAEL (NOEL)	2200 ppm (both immediately post-exposure and after 26 wk recovery)
LOAEL (LOEL)	7000 ppm (both immediately post-exposure and after 26 wk recovery)
Remarks	The only effect observed in the male rats after 26 weeks of exposure was interstitial cell hyperplasia of the testis (1 0/1 0) in the 7000 ppm group; following the 26-week recovery period the only effect in rats was a marginal increase in benign testicular interstitial cell tumors (9/30 at 7000 ppm).
Results c e)	Spinal cord degeneration / nasal turbinates degeneration
NOAEL (NOEL)	2200 ppm (post exposure) (<70 ppm after 26 wk recovery) / 2200 ppm (post exposure) (70 ppm after 26 wk recovery)
LOAEL (LOEL)	7000 ppm (post exposure) (70 ppm after 26 wk recovery) / 7000 ppm (post exposure) (220 ppm after 26 wk recovery)
Remarks	Survival of mice was reduced in the 7000 ppm group; early deaths were attributed to variou: neoplastic lesions and moribund sacrifices due to hindlimb paralysis. In male mice, incidences of malignant neoplastic lesions in the liver, lung, forestomach, and harderian glar were significantly increased following the 26-week exposure and 26-week recovery periods 700 ppm and higher exposures. Non-neoplastic lesions were observed in male mice exposec to isoprene and included spinal cord degeneration (≥70 ppm) and degeneration of the olfactory epithelium (≥220 ppm). Slight increases in testicular atrophy, epithelial hyperplasi: of the forestomach, partial hindlimb paralysis and a nonresponsive macrocytic anemia were also seen in male mice.

	Selected non-neoplastic lesions in mice were as follows (0, 70, 220, 700, 2200, 7000 ppm) - After 26 weeks exposure; Nasal turbinates/olfactory epithelial degeneration - 0/10, 0/10, 0/10, 1/10, 1/10, 10/10. Testes/atrophy - 0110, 0/10, 0/10, 0/10, 1110, 5110. Spinal cord/degeneration - 0110, O/IO, 0/10, 0/10, 1/10, 10/10. After 26 weeks recovery: Nasal turbinates/olfactory epithelial degeneration - 1130, 2/30, 5/29, 11/30, 25/30, 28/28. Testes/atrophy - 0130, 0130, 0/29, 0/30, 0130, 3/29. Spinal cord/degeneration - 4130, 20/30, 19/29, 17/29, 13/28..
Conclusions (study authors)	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 (7000 ppm).
Conclusions (contractor)	Uncertainty has arisen on the reliability of the reported spinal cord degeneration data reported in this study. Re-examination of the spinal slides from the NTP study by a neuropathologist (Garman, 2001) has discerned a lack of isoprene-related spinal lesions in the 26-week recovery mice. Spinal lesions observed in mice were not different in appearance or incidence in exposed groups versus the control animals. These findings call into question the conclusion that the NOAEL for this study is less than 70 ppm.
Quality Reliabilities	Reliable with restrictions. Comparable to guideline studies. This study involved exposures of male rats and male mice to isoprene for 6 months, therefore provided additional data on repeated dose toxicity and carcinogenicity.
References	Melnick RL; Sills RC; Roycroft JH; Chou BJ; Ragan HA; Miller RA (1994). Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Res. 54:5333-5339. National Toxicology Program (1989). Inhalation Developmental Toxicology Studies: Teratology Study of lsoprene in Mice and Rats. TER88045; NTIS#DE89008095 Garman, Robert H. (2001). Supplementary Neuropathology Review. NTP Isoprene Stop-Exposure Study, Toxicity Report Series No. 3 1. Unpublished report for International Institute of Synthetic Rubber Producers, Inc.
Other Last changed	07-Sep-01 Robust summary prepared by a contractor to the Panel.

Repeated Dose Toxicity

Test Substance	
Remarks	Isoprene, CAS# 78-79-5 Purity >99.7%.
Method	
Method/guideline followed	Other
Test type	2-year carcinogenicity study.
GLP	Yes.
Year	1997.
Species	Rat.
Strain	Fisher 344.
Route of administration	Inhalation (vapor).
Duration of test	104 weeks.
Doses/concentration levels	0, 220, 700, or 7000 ppm.
Sex	50 male, 50 female per group.
Exposure period	6 hours/day.
Frequency of treatment	5 days/week for 104 weeks.
Control group and treatment	50 male, 50 female, exposed to air only.
Post exposure observation period	None.
Statistical methods	Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Urine data was analyzed by nonparametric methods.
Test Conditions	Groups of 50 rats/sex /group (approx. 6 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 104 weeks. Individual clinical observations were recorded initially, monthly through week 89, and then every 2 weeks until the end of the study. Individual body weights were recorded initially, monthly through week 91, and then every 2 weeks until the end of the study. Urine samples were collected 3, 6, 12, and 18 months from 10 rats/sex/group and analyzed for urine weight, creatinine, and vinyl lactic acid (a metabolite of isoprene). After 104 weeks of exposure, necropsies were performed on all rats and all major tissues preserved. Histopathologic examinations were performed on all tissues from all study animals. No blood analyses or organ weights were performed.
Results	
NOAEL (NOEL)	Not determined
LOAEL (LOEL)	Not determined
Remarks	Survival of all exposed groups was similar to the chamber controls. There were no exposure-related changes in clinical observations or body weights. The incidences of mammary gland fibroadenoma in 7,000 ppm males (7/50) and in all groups of exposed females (12150, 19/50, 17150) were significantly greater than those in the chamber control groups (1150 males, 7150 females). The incidences of renal tubule adenoma in 7,000 ppm males (6150) and of renal tubule hyperplasia in 700 ppm and 7,000 ppm males (6/50, 8150) were significantly greater than those in the chamber controls (0150). The severity of kidney nephropathy was slightly increased in 7,000 ppm males when compared to chamber controls. An exposure-related increase in the incidences of interstitial cell adenoma of the testis was observed in male rats (33/50, 37/50, 44/50, 48/50). The incidences of bilateral interstitial cell adenoma and of unilateral and bilateral interstitial cell adenoma (combined) of the testis in the 700 ppm and 7,000 ppm (37/50, 48/50) males were significantly greater than in the chamber controls (20/50). Single incidences of several rare neoplasms including benign astrocytoma, malignant glioma, malignant medulloblastoma, benign meningeal granular cell tumor, and meningeal sarcoma were observed in the brains of female rats in all three exposure groups. The incidences of splenic fibrosis in the 700 and 7,000 ppm males (24/50, 22/50) were significantly greater than that in the chamber control group (11150).
Conclusions (contractor)	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.
Conclusions (study authors)	There was clear evidence of tumorigenic activity in male rats based on increased incidences of mammary gland tibroadenoma and carcinoma, renal tubule adenoma, and testicular interstitial cell adenoma. There was some evidence of carcinogenic activity in female 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.
Data Quality Reliabilities	Reliable without restrictions.
References	National Toxicology Program (1997). Toxicology and Carcinogenesis Studies of Isoprene (CAS No. 78-79-S) in F344/N Rats (Inhalation Studies), Report No. TR-486.
Other Last changed	07-Sep-0 1 Robust summary prepared by a contractor to the Panel.

Repeated Dose Toxicity

Test Substance	Isoprene, CAS# 78-79-5
Remarks	Purity >99.0%.
Method	
Method/guideline followed	Other
rest type	2-year carcinogenicity study.
GLP	Yes.
Year	1996.
Species	Mouse.
Strain	$B6C3F_1$.
Route of administration	Inhalation (vapor).
Duration of test	105 weeks.
Doses/concentration levels	0, 10, 70, 140, 280, 700, 2200 ppm.
Sex	50 male, 50 female per group.
Exposure period	4 or 8 hours/day.
Frequency of treatment	Variable - 5 days/week for 20, 40, or 80 weeks.
Control group and treatment	50 male, 50 female, exposed to air only.
Post exposure observation period	Variable - animals held following exposures until week 96 or 105.
Statistical methods	Body weights, organ weights and hematology data were evaluated by analysis of variance (ANOVA) followed by Duncan's new multiple range test. lncidences of tumor types were analyzed using Fischer's exact test applied to each combination of exposure group and tumor type.
Test Conditions	Twelve groups of 50 male mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period until week 105. Three groups of 50 female mice were exposed to 0, 10, and 70 ppm for 8 hours/day for 80 weeks and also held for observation until week 105. Clinical observations and body weights were recorded weekly for 13 weeks and then monthly. Hematology and micronucleus evaluations were performed on 10 mice/group at 40 and 80 weeks. Complete histopathology evaluations were performed on organs and tissues from all mice.
Results	
NOAEL (NOEL)	10 ppm
LOAEL (LOEL)	70 ppm
Remarks	The carcinogenic potential of isoprene was evaluated as a function of concentration, length of daily exposure, and weeks of exposure as independent variables. Exposure of mice to the varied concentrations and schedules did not produce any significant signs of general toxicity. There was a concentration-related effect on survival due to increases in selected tumor development and associated mortality. Survival was near or below 50% after 95 weeks for mice exposed >280 ppm for 80 weeks - surviving mice in these groups were necropsied during week 96. Isoprene exposure caused an increase in neoplasms of the lung, liver, Harderian gland, forestomach, lymphoreticular system of male mice and in the Harderian gland and pituitary gland of female mice at concentrations of 70 ppm and higher. The product of concentration and length/duration of exposure was not a sufficient basis for prediction of tumor risk. In the micronucleus evaluation, the mean incidence of micronuclei in peripheral blood was significantly increased at 700 ppm and higher after 80 weeks, and at 2200 ppm after 40 weeks (the 280 and 700 ppm groups were not sampled by protocol design).
Conclusions (study authors)	The results of this study indicated that concentration, length of daily exposure, and weeks of exposure did not affect tumor incidence equivalently and total cumulative exposure was not sufficient for predicting oncogenic risk from isoprene exposure in mice. There appeared to be threshold for oncogenic effects in mice, which varied by organ and tumor type. For male mice, the LOEL was 700ppm for lung tumor and hemangiosarcoma, 280 ppm for malignant forestomach tumors and histiocytic sarcomas, 140 ppm for liver tumors, and 70 pmm for Harderian gland tumors. For female mice, the LOEL was 70 ppm for total

	non-liver, non-lung adenomas and possibly for hemagiosarcomas.
Data Quality	
Reliabilities	Reliable without restrictions.
References	Placke ME, Griffis L, Bird M, Bus J, Persing RL, and Cox LA Jr (1996). Chronic inhalation oncogenicity study of isoprene in B6C3F1 mice. Toxicology 113:253-62.
Other	
Last changed	07-Sep-01 Robust summary prepared by a contractor to the Panel.

Developmental Toxicity/Teratogenicity

rest Substance	soprene, CAS# 78-79-5
Remarks	Purity 199.7%.
Method	
Method/guideline followed	3ECD 414
Test type	Developmental toxicity (teratogenicity) study.
GLP	Yes.
Year	1989.
Species	Rat and mouse.
Strain	Sprague-Dawley (rat) and CD- l/Swiss (mouse).
Route of administration	nhalation (vapor).
Concentration levels	1,280, 1400, or 7000 ppm.
Sex	-30 pregnant females per group; plus 10 virgin females per group for comparison.
Exposure period	Gestation days 6-19 (rats) or 6- 17 (mice).
Frequency of treatment	6 hours/day.
Control group and treatment	Air-exposed only.
Duration of test	Females sacrificed on gestation day 20 (rats) or 18 (mice).
Statistical methods	Not specified.
Remarks for Test Conditions.	Positively mated mice were exposed on days 6-17 of gestation and rats on days 6-19. The lay of plug or sperm detection was designated as day 0. Body weights were recorded throughout the study period, and uterine and fetal body weights were obtained at sacrifice. Implants were enumerated and their status recorded. Live fetuses were sexed and examined For gross, visceral, skeletal, and soft-tissue craniofacial defects.
Results	
NOAEL maternal toxicity	7000 ppm (rats), 1400 ppm (mice).
NOAEL developmental toxicity	7000 ppm (rats), <280 ppm (mice).
Maternal effects	Exposure of pregnant rats to these concentrations of isoprene did not result in apparent maternal toxicity. The only effect observed in the rat dams was an increased kidney to body weight ratio at the highest level (7000 ppm). Exposure of Swiss (CD-l) mice to isoprene resulted in (from day 12 onward) significant reductions in maternal body weight, body weight gain during treatment, and uterine weight for the 7000 ppm group. Liver to body weight ratios for pregnant mouse dams were significantly increased in the 1400 and 7000 ppm groups compared to the control group, and kidney to body weight ratios were significantly increased in the 7000 ppm group.
Embryo/fetal effects	In rats, there was no adverse effect on any reproductive index at any level and there was no increase in fetal malformations or variations. A slight, but not statistically significant, increase in the incidence of reduced vertebral ossifications (centra) was noted at 7000 ppm. In mice, there was an exposure-related and statistically significant reduction in fetal body weights at the 280 ppm level for female fetuses and at the 1400 ppm level for male fetuses. No embryotoxicity in the form of increased intrauterine death was present at any exposure level. There was no significant increase in the incidence of fetal malformations or variations, although two fetuses with cleft palate were found, one in each of the two highest exposure groups (1400 and 7000 ppm). Cleft palates were not detected in the control group. An increased incidence of supernumerary ribs was observed at 7000 ppm, although this skeletal variation is generally considered a secondary effect of maternal toxicity or stress and its significance is unclear.
Conclusions (study authors)	Pregnant Sprague-Dawley rats and their offspring exhibited no significant toxic effects of isoprene at any exposure level in this study. Swiss (CD-l) mouse dams exhibited significant toxic effects only at the 7000 ppm level; however the offspring exhibited significant signs of toxicity, including reductions in fetal body weight at all exposure concentrations.
Data Quality *Reliabilities*	Reliable without restrictions. NTP-sponsored study.

References	National Toxicology Program (1989). Inhalation Developmental Toxicology Studies: Teratology Study of Isoprene in Mice and Rats. TER88045; NTIS#DE89008095.
Other	
Last *changed*	07-Sep-01 Robust summary prepared by a contractor to the Panel.

Robust Summary - Group 3: C5 Non-Cyclics

Toxicity to Reproduction

Test Substance	soprene, CAS# 78-79-5
Remarks	Purity >99%.
Method	
Method/guideline followed	Other.
Test type	Satellite groups from 13 -week or 26-week inhalation exposure.
GLP	Yes.
Year	1994.
Species	Rat and mouse.
Strain	F344 rats and B6C3F1 mice.
Route of administration	Inhalation (vapor).
Duration of test	13 weeks.
Concentration levels	3, 70,700, or 7000 ppm.
Sex	10 male, 10 female per group.
Exposure period	6 hoursday.
Frequency of treatment	5 daysiweek.
Control group and treatment	10 male, 10 female, air-only exposed.
Statistical methods	Analysis of incidence of neoplastic and nonneoplas tic lesions was performed.
Remarks for Test Conditions.	Groups of 10 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Sperm motility, vaginal cytology, and histopathologic evaluations of the reproductive organs were performed on all rats and mice as part of the terminal sacrifice for the core 13-week subchronic inhalation study.
Results	
NOAEL	2200 ppm (rats). 220 ppm (mice).
	There were no exposure -related effects in rats except a slight increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7000 ppm group. In mice, testicular weight was reduced 35% in the 7000 ppm group, and morphological changes (seminiferous tubular atrophy) were detected in 2/10 mice. Males in the 700 and 7000 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 7000 ppm exhibited estrous cycle lengths significantly longer than the control group (4.8 vs. 4.2 days).
Conclusions (contractor)	No significant effects on reproductive endpoints were observed in rats except slight changes in the testis at the highest exposure level (7000 ppm). Mice 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.
Data Quality *Reliabilities*	Reliable with restrictions. Limited reproductive toxicity data obtained as part of a NTP-sponsored subchronic inhalation toxicity study.
References	Melnick RL; Sills RC; Roycroft JH; Chou BJ; Ragan HA; Miller RA (1994). Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Res. 54:5333-5339.
Other	
Last changed	07-Sep-01 Robust summary prepared by a contractor to the Panel.

AR 201 - 13186 B

Fish Acute Toxicity

Test Substance:	CAS No. 10466-O; n-Pentane
Method/Guideline:	OECD 203
Year (guideline):	1992
Type (test type):	Semistatic Fish Acute Toxicity Test
GLP:	Yes
Year (study performed):	1997
Species:	Rainbow Trout (Oncorhynchus mykiss)
Analytical Monitoring:	Yes
Exposure Period:	96 hour
Statistical Method: (FT ▪ ME)	Trimmed Spearman Karber Method.
Test Conditions: (FT ▪ TC) . **Note: Concentration prep., vessel** type, **volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, weight, loading.**	The test material was added directly to the test chambers via injection by syringe. Test vessels were 4L aspirator bottles completely filled with solution. Control and dilution water were a laboratory blend of filtered well water and reverse osmosis water. Test vessels were sealed with no headspace. Test solutions were mixed gently using a magnetic stir bar for the duration of the test. Test solutions were renewed daily by siphoning out 80% and refilling with dilution water and redosing. Each test vessel contained 5 fish. Three replicates were prepared per treatment, two with fish and one for sampling purposes. Nominal n-pentane treatment levels were 3.12, 6.25, 12.5, 25, and 50mg/L, which measured 0.635, 1.02, 2.21, 5.81, and 7.03mg/L, respectively, and are based on the mean of test material in samples taken from the new and old solutions. Test temperature was 13.6 Deg C. Lighting was 860Lux with 16 hrs light and 8 hrs dark. Dissolved Oxygen was 8.3 to 10.8 mg/L for "new" solutions and 6.9 to 9.2 mg/L for "old" solutions. The pH ranged from 7.0 to 7.2 for "new" solutions and 7.0 to 7.2 for "old" solutions. Fish supplied by Thomas Fish Co.; age=5 weeks old; mean wt.=0.249g; mean total length=3.4cm; test loading=0.277g fish/L.
Results: (FT ▪ RS) **Units/Value:**	96 hour LC50 = 4.26mg/L (95% CI 3.6 to 5.04mg/L) based upon measured values of old and new solutions.
. **Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.**	Analytical method used was Headspace Gas Chromatography with Flame Ionization Detection (GC-FID). The fish were slightly shorter than the guideline suggestion of 4.0 to 6.0cm, which were purposely selected to help maintain oxygen levels in the closed system. Fish size had no significant effect on study outcome.

RESULTS con't:

Measured Conc. (mg/L)	Fish Total Mortalitv (@96 hrs)*
Control	0
0.635	0
1.02	0
2.21	0
5.81	7
7.03	10

* 10 fish added at test initiation

Conclusion: (FT ▪ CL)

Reliability: (FT ▪ RL) (1) Reliable without restriction

Reference: (FT ▪ RE) Exxon Biomedical Sciences, Inc. 1997. Acute Fish Toxicity Test with Rainbow Trout. Study #157558.

Other (source): (FT ▪ SO)

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FT - Freetext
ME ▪ Method
TC - Test Conditions
RS - Results
CL - Conclusion
RL ▪ Reliability
RE - Reference
SO - Source

AR201-13186B

Fish Acute Toxicity

Test Substance:	CAS No. 68526-52-3; Alkenes, C6 Rich
Method/Guideline:	OECD 203
Year (guideline):	1992
Type (test type):	Semistatic Fish Acute Toxicity Test
GLP:	Yes
Year (study performed):	1995
Species:	Rainbow Trout (Oncorhynchus mykiss)
Analytical Monitoring:	Yes
Exposure Period:	96-hour
Statistical Method: (FT - ME)*	Trimmed Spearman-Karber Method (Hamilton, M.A. et al. 1977. Trimmed Spearman-Karber Method for Estimating Median Lethal Concentration in Toxicity Bioassays. Environ. Sci. Technol. 11:714-719.)
Test Conditions: (FT - TC) . **Note: Test material loading preparation, vessel type, volume, replication, water quality parameters, environmental conditions, and test organism supplier, age, size, weight, and loading.**	Each test solution was prepared by adding the test substance, via syringe, to 19.5 L of laboratory blend water in 20 L glass carboys. The solutions were mixed for 24 hours with a vortex of (10%. Mixing was performed using a magnetic stir plate and Teflon@ coated stir bar at room temperature (approximately 22C). After mixing, the solutions were allowed to settle for one hour after which the Water Accommodated Fraction (WAF) was siphoned from the bottom of the mixing vessel through a siphon that was placed in the carboy prior to adding the test material. Test vessels were 4.0 L aspirator bottles that contained approximately 4.5 L of test solution. Each vessel was sealed with no headspace after 5 fish were added. Three replicates of each test material loading were prepared. Approximately 80% of each solution was renewed daily from a freshly prepared WAF. Test material loading levels included: 6.25, 12.5, 25, 50, and 100 mg/L, which measured 2.9, 6.6, 13.4, 16.9, and 44.0 mg/L, respectively, and are based on the mean of samples taken from the new and old test solutions. A control containing no test material was included and the analytical results were below the quantitation limit, which was 0.2 mg/L. Analytical method used was Headspace Gas Chromatography with Flame Ionization Detection (GC-FID). Test temperature was 16C (sd = 0.04). Lighting was 623 to 629 Lux with a 16hr light and 8-hr dark cycle. Dissolved oxygen ranged from 7.7 to 9.6 mg/L for "new" solutions and 4.5 to 7.5 mg/L for "old" solutions. The pH ranged from 8.2 to 8.5 for "new" solutions and 7.2 to 7.7 for "old" solutions. Fish supplied by Thomas Fish Co. Anderson, CA, USA; age at test initiation = approximately 5 weeks; mean wt. at test termination = 0.375 g; mean total length at test termination = 3.6 cm; test loading

= 0.42 g of fish/L. The fish were slightly shorter than the guideline suggestion of 4.0 to 6.0 cm, which were purposely selected to help maintain oxygen levels in the closed system. Fish size had no significant effect on study outcome.

Results: (FT ▪ RS)

Units/Value:

96-hour LL50 = 12.8 mg/L (95% Cl 10.7 to 15.3 mg/L) based upon loading rates.

. **Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.**

Loading Rate (ma/L\	Fish Total Mortalitv (@96 hrs)*
Control	0
6.25	**0**
12.5	7
25	15
50	15
100	15

* 15 fish added at test initiation

Conclusion: (FT - CL)

Reliability: (FT ▪ RL)

(1) Reliable without restriction

Reference: (FT ▪ RE)

Exxon Biomedical Sciences, Inc. 1996. Fish, Acute Toxicity Test. Study #1 19058. Exxon Biomedical Sciences, Inc., East Millstone, NJ, USA.

Other (source): (FT ▪ SO)

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* IUCLID field abbreviations include:
FT ▪ Freetext
ME - Method
TC - Test Conditions
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