

APPENDIX II

ROBUST SUMMARIES OF STUDIES USED TO CHARACTERIZE THE
C5 NON-CYCLICS CATEGORYPHYSICO-CHEMICAL ROBUST SUMMARIES

Melting Point

Test Substance	Other TS [CAS # 513-35-9; 64742-83-2; 68410-97-9; 68476-43-7; 68476-55-1; 68477-35-0; 68514-39-6; 68527-11-7; 68527-19-5; 68603-00-9; 68603-03-2; 68606-29-1; 68606-36-0; 68956-55-8; 78-79-5]
Method/Guideline	Calculated values using MPBPWIN version 1.40, a subroutine of the computer program EPIWIN version 3.04
Year (guideline)	1999
Type (test type)	Not applicable
GLP	Not applicable
Year (study performed)	Not applicable
Test Conditions • Note: Concentration prep., vessel type, replication, test conditions.	<p>Melting Point is calculated by the MPBPWIN subroutine, which is based on the average result of the methods of K. Joback and Gold and Ogle.</p> <p>Joback's Method is described in Joback, K.G. 1982. A Unified Approach to Physical Property Estimation Using Multivariate Statistical Techniques. In <u>The Properties of Gases and Liquids</u>. Fourth Edition. 1987. R.C. Reid, J.M. Prausnitz and B.E. Poling, Eds.</p> <p>The Gold and Ogle Method simply uses the formula $T_m = 0.5839T_b$, where T_m is the melting point in Kelvin and T_b is the boiling point in Kelvin.</p>
Results Units/Value: • Note: Deviations from protocol or guideline, analytical method.	<p>Calculated and measured melting point data for representative constituents of the C5 Non-Cyclics Category are listed below. The data identify a potential melting point range for substances represented by the 15 CAS numbers under <u>Test Substance</u>. Substances in this category do not have a specific melting point value. Actual melting point ranges for substances in this category will vary dependent on their constituent composition.</p>

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	<p>Commercial substances in this category consist of both high purity hydrocarbons and complex hydrocarbon reaction products with a carbon number distribution that is predominantly C5. The nine chemicals selected to represent the melting point range of this category are C5 hydrocarbons that can be found in substances identified by the 15 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category name, chemistry/structure, measured boiling point ranges for category substances, olefinic process (distillation) knowledge, and percentage of the composition of the represented process streams.</p> <table border="1"> <thead> <tr> <th>Substance <u>Constituent</u></th> <th>Calculated <u>MP (°C)</u></th> <th>Measured* <u>MP (°C)</u></th> </tr> </thead> <tbody> <tr> <td>cis-butene-2</td> <td>-120.4</td> <td>-105.5</td> </tr> <tr> <td>cis-pentene-2</td> <td>-107.1</td> <td>-140.2</td> </tr> <tr> <td>3-methyl-1-butene</td> <td>-120.5</td> <td>-168.5</td> </tr> <tr> <td>1,4-pentadiene</td> <td>-109.8</td> <td>-148.8</td> </tr> <tr> <td>Isopentane</td> <td>-119.0</td> <td>-159.9</td> </tr> <tr> <td>Isoprene</td> <td>-118.9</td> <td>-145.9</td> </tr> <tr> <td>n-pentane</td> <td>-106.9</td> <td>-129.7</td> </tr> <tr> <td>2-methyl-2-butene</td> <td>-116.2</td> <td>-133.7</td> </tr> <tr> <td>cyclopentene</td> <td>-93.2</td> <td>-135.1</td> </tr> </tbody> </table> <p>* Experimental values from EPIWIN database. The data represent a potential melting point range for substances represented by the 15 CAS numbers under <u>Test Substance</u>.</p>	Substance <u>Constituent</u>	Calculated <u>MP (°C)</u>	Measured* <u>MP (°C)</u>	cis-butene-2	-120.4	-105.5	cis-pentene-2	-107.1	-140.2	3-methyl-1-butene	-120.5	-168.5	1,4-pentadiene	-109.8	-148.8	Isopentane	-119.0	-159.9	Isoprene	-118.9	-145.9	n-pentane	-106.9	-129.7	2-methyl-2-butene	-116.2	-133.7	cyclopentene	-93.2	-135.1
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	<p>C5 Non-Cyclics Category substances arise from production processes associated with ethylene manufacturing. The 15 CAS numbers are used to describe the ten process streams arising from the ethylene process and other associated C5 processes. The process streams in this category consist of high purity hydrocarbons or complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes and predominantly non-cyclic.</p> <p>More information on the C5 Non-Cyclics Category can be found in the American Chemistry Council, Olefins Panel test plan for this category (1).</p> <ol style="list-style-type: none"> 1. Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.
Conclusion	<p>Based on calculated constituent data, substances in this category can have a melting range of -93.2 to -120.5 °C. Based on measured constituent data, substances in this category can have a melting range of -105.5 to -168.5°C.</p>
Reliability	<p>(2) Reliable with restrictions</p> <p>The results include calculated data based on chemical structure as modeled by EPIWIN and measured data for specific chemicals as cited in the EPIWIN database. The data represent a potential melting point range for substances represented by the 15 CAS numbers listed under <u>Test Substance</u>. This robust summary has a reliability rating of 2 because the data are not for specific substances in the C5 Non-Cyclics Category, but rather for selected constituents. These selected constituents represent all substances defined by this category and as such, this robust summary represents a "key study" for melting point range based on constituent data.</p>
Reference	<p>EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA. (Melting point values were calculated by the MPBPWIN subroutine and measured data came from the database in the computer program.)</p>

Boiling Point

Test Substance	Other TS [CAS # 513-35-9; 64742-83-2; 68410-97-9; 68476-43-7; 68476-55-1; 68477-35-0; 68514-39-6; 68527-11-7; 68527-19-5; 68603-00-9; 68603-03-2; 68606-29-1; 68606-36-0; 68956-55-8; 78-79-5]
Method/Guideline	Calculated values using MPBPWIN version 1.40, a subroutine of the computer program EPIWIN version 3.04
Year (guideline)	1999
Type (test type)	Not applicable
GLP	Not applicable
Year (study performed)	Not applicable
Estimation Pressure	760 mm Hg
Test Conditions <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, test conditions. 	Boiling Point is calculated by the MPBPWIN subroutine, which is based on the calculation method of S. Stein and R. Brown in "Estimation of Normal Boiling Points from Group Contributions". 1994. J. Chem. Inf. Comput. Sci. 34: 581-587.
Results Units/Value: <ul style="list-style-type: none"> Note: Deviations from protocol or guideline, analytical method. 	<p>Calculated and measured boiling point data for representative constituents of the C5 Non-Cyclics Category are listed below. The data identify a potential boiling point range for substances represented by the 15 CAS numbers under <u>Test Substance</u>. Substances in this category do not have a specific boiling point value. Actual boiling point ranges for substances in this category will vary dependent on their constituent composition.</p> <p>Commercial substances in this category consist of both high purity hydrocarbons and complex hydrocarbon reaction products with a carbon number distribution that is predominantly C5. The nine chemicals selected to represent the boiling point range of this category are C5 hydrocarbons that can be found in substances identified by the 15 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category name, chemistry/structure, measured boiling point ranges for category substances, olefinic process (distillation) knowledge, and percentage of the composition of the represented process streams.</p>

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	<p>1. Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.</p>
Conclusion	<p>Based on calculated constituent data, substances in this category can have a boiling range of 27.82 to 65.86°C @ 760 mm Hg. Based on measured constituent data, substances in this category can have a boiling range of 0.8 to 44.2°C @ 760 mm Hg.</p>
Reliability	<p>(2) Reliable with restrictions</p> <p>The results include calculated data based on chemical structure as modeled by EPIWIN and measured data for specific chemicals as cited in the EPIWIN database. The data represent a potential boiling point range for substances represented by the 15 CAS numbers listed under <u>Test Substance</u>. This robust summary has a reliability rating of 2 because the data are not for specific substances in C5 Non-Cyclics Category, but rather for selected constituents. These selected constituents represent all substances defined by this category and as such, this robust summary represents a "key study" for boiling point range based on constituent data.</p>
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Boiling Point

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Test type GLP Year</p> <p><u>Results</u> Boiling Point <u>Remarks</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Naphthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was stable for the duration of the study.</p> <p>OECD Method 103 Boiling temperature Yes 2002 23.5°C to 52.0°C Main fractions at 27.5°C and 40.5°C The test substance boils over a temperature range of 23.5°C to 52°C and exhibits two main boiling temperatures of 27.5°C and 40.5°C.</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS/034. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Boiling Point

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Test type GLP Year</p> <p><u>Results</u> Boiling Point <u>Remarks</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s).</p> <p>Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8.</p> <p>The three major components analysed were shown to be stable for the duration of the study.</p> <p>OECD Method 103 Boiling temperature Yes 2002 25.0°C to 56.5°C Main components at 37.0°C and 47.0°C</p> <p>The test substance has two main boiling temperatures of 36.8°C and 46.9°C where a continuous string of bubbles is observed. Apart from these temperatures, between 24.8°C and 56.5°C a slow release of bubbles (one at a time) was observed coming from the tube.</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS035. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Partition Coefficient

Test Substance	Other TS [CAS # 513-35-9; 64742-83-2; 68410-97-9; 68476-43-7; 68476-55-1; 68477-35-0; 68514-39-6; 68527-11-7; 68527-19-5; 68603-00-9; 68603-03-2; 68606-29-1; 68606-36-0; 68956-55-8; 78-79-5]
Method/Guideline	Calculated values using KOWWIN version 1.65, a subroutine of the computer program EPIWIN version 3.04
Year (guideline)	1999
Type (test type)	Not applicable
GLP	Not applicable
Year (study performed)	Not applicable
Estimation Temperature	25°C
Test Conditions	Octanol / Water Partition Coefficient is calculated by the KOWWIN subroutine, which is based on an atom/fragment contribution method of W. Meylan and P. Howard in "Atom/fragment contribution method for estimating octanol-water partition coefficients". 1995. <i>J. Pharm. Sci.</i> 84:83-92.
Results Units/Value: <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, test conditions. 	<p>Calculated and measured log K_{ow} data for representative constituents of the C5 Non-Cyclics Category are listed below. The data identify a potential log K_{ow} range for substances represented by the 15 CAS numbers under <u>Test Substance</u>. Substances in this category do not have a specific log K_{ow} value. Actual log K_{ow} ranges for substances in this category will vary dependent on their constituent composition.</p> <p>Commercial substances in this category consist of both high purity hydrocarbons and complex hydrocarbon reaction products with a carbon number distribution that is predominantly C5. The nine chemicals selected to represent the log K_{ow} range of this category are C5 hydrocarbons can be found in substances identified by the 15 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category name, chemistry/structure, measured boiling point ranges for category substances, olefinic process (distillation) knowledge, and percentage of the composition of the represented process streams.</p>
<ul style="list-style-type: none"> Note: Deviations from protocol or guideline, analytical method. 	

	Substance <u>Constituent</u>	Calculated <u>log K_{ow} @ 25°C</u>	Measured* <u>log K_{ow} @ 25°C</u>																														
	cis-butene-2	2.09	2.31																														
	cis-pentene-2	2.58	na																														
	3-methyl-1-butene	2.59	na																														
	1,4-pentadiene	2.52	2.48																														
	Isopentane	2.72	na																														
	Isoprene	2.58	2.42																														
	n-pentane	2.80	3.39																														
	2-methyl-2-butene	2.64	2.67																														
	cyclopentene	2.47	na																														
	* Experimental values from EPIWIN database. na = not available The data represent a potential log K _{ow} range for substances represented by the 15 CAS numbers under <u>Test Substance</u> .																																
Test Substance	<p>The C5 Non-Cyclics Category includes the following CAS numbers:</p> <table> <tbody> <tr> <td>513-35-9</td> <td>2-Butene, 2-methyl-</td> </tr> <tr> <td>64742-83-2</td> <td>Naphtha, petroleum, light steam-cracked</td> </tr> <tr> <td>68410-97-9</td> <td>Distillates, petroleum, light distillate hydrotreating process, low-boiling</td> </tr> <tr> <td>68476-43-7</td> <td>Hydrocarbons, C4-6, C5-rich</td> </tr> <tr> <td>68476-55-1</td> <td>Hydrocarbons, C5-rich</td> </tr> <tr> <td>68477-35-0</td> <td>Distillates, petroleum, C3-6, piperylene-rich</td> </tr> <tr> <td>68514-39-6</td> <td>Naphtha, petroleum, light steam-cracked, isoprene-rich</td> </tr> <tr> <td>68527-11-7</td> <td>Alkenes, C5</td> </tr> <tr> <td>68527-19-5</td> <td>Hydrocarbons, C1-4, debutanizer fraction</td> </tr> <tr> <td>68603-00-9</td> <td>Distillates, petroleum, thermal cracked naphtha and gas oil</td> </tr> <tr> <td>68603-03-2</td> <td>Distillates, petroleum, thermal cracked naphtha and gas oil, extractive</td> </tr> <tr> <td>68606-29-1</td> <td>Hydrocarbons, C4 and C8, butene concentrator by-product</td> </tr> <tr> <td>68606-36-0</td> <td>Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product</td> </tr> <tr> <td>68956-55-8</td> <td>Hydrocarbons, C5-unsatd.</td> </tr> <tr> <td>78-79-5</td> <td>1,3-Butadiene, 2-methyl-</td> </tr> </tbody> </table> <p>C5 Non-Cyclics Category substances arise from production processes associated with ethylene manufacturing. The 15 CAS numbers are used to describe the ten process streams arising from the ethylene process and other associated C5 processes. The process streams in this category consist of high purity hydrocarbons or complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes and predominantly non-cyclic.</p> <p>More information on the C5 Non-Cyclics Category can be found in the American Chemistry Council, Olefins Panel test plan for this category (1).</p>			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	68476-55-1	Hydrocarbons, C5-rich	68477-35-0	Distillates, petroleum, C3-6, piperylene-rich	68514-39-6	Naphtha, petroleum, light steam-cracked, isoprene-rich	68527-11-7	Alkenes, C5	68527-19-5	Hydrocarbons, C1-4, 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	68606-29-1	Hydrocarbons, C4 and C8, butene concentrator by-product	68606-36-0	Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product	68956-55-8	Hydrocarbons, C5-unsatd.	78-79-5	1,3-Butadiene, 2-methyl-
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	<p>1. Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.</p>
Conclusion	<p>Based on calculated constituent data, substances in this category can have a log K_{ow} range of 2.09 to 2.80 @ 25°C. Based on measured constituent data, substances in this category can have a log K_{ow} range of 2.31 to 3.39 @ 25°C.</p>
Reliability	<p>(2) Reliable with restrictions</p> <p>The results include calculated data based on chemical structure as modeled by EPIWIN and measured data for specific chemicals as cited in the EPIWIN database. The data represent a potential log K_{ow} range for substances represented by the 15 CAS numbers under <u>Test Substance</u>. This robust summary has a reliability rating of 2 because the data are not for specific substances in the C5 Non-Cyclics Category, but rather for selected constituents. These selected constituents represent all substances defined by this category and as such, this robust summary represents a "key study" for log K_{ow} range based on constituent data.</p>
Reference	<p>EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA. (Log K_{ow} values were calculated by the KOWWIN subroutine and measured data came from the database in the computer program.)</p>

Partition Coefficient

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Test type GLP Year</p> <p><u>Results</u> Partition coefficient</p> <p><u>Remarks</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Naphthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was stable for the duration of the study.</p> <p>OECD Method 117 (HPLC Method) Partition coefficient Yes 2002</p> <p>log P = a range between 2.64 and 4.21 at 21.5°C</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS/034. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Partition Coefficient

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Test type GLP Year</p> <p><u>Results</u> Partition coefficient</p> <p><u>Remarks</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s).</p> <p>Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8.</p> <p>The three major components analysed were shown to be stable for the duration of the study.</p> <p>OECD Method 117 (HPLC Method) Partition coefficient Yes 2002</p> <p>log P = a range between 3.19 and 3.25 at 21°C</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS035. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Vapor Pressure

Test Substance	Other TS [CAS # 513-35-9; 64742-83-2; 68410-97-9; 68476-43-7; 68476-55-1; 68477-35-0; 68514-39-6; 68527-11-7; 68527-19-5; 68603-00-9; 68603-03-2; 68606-29-1; 68606-36-0; 68956-55-8; 78-79-5]
Method/Guideline	Calculated values using MPBPWIN version 1.40, a subroutine of the computer program EPIWIN version 3.04
Year (guideline)	1999
Type (test type)	Not applicable
GLP	Not applicable
Year (study performed)	Not applicable
Estimation Temperature	25°C
Test Conditions <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, test conditions. 	<p>Vapor Pressure is calculated by the MPBPWIN subroutine, which is based on the average result of the methods of Antoine and Grain. Both methods use boiling point for the calculation.</p> <p>The Antoine Method is described in the <u>Handbook of Chemical Property Estimation</u>. Chapter 14. W.J. Lyman, W.F. Reehl and D.H. Rosenblatt, Eds. Washington, D.C.: American Chemical Society. 1990.</p> <p>A modified Grain Method is described on page 31 of Neely and Blau's <u>Environmental Exposure from Chemicals</u>, Volume 1, CRC Press. 1985.</p>
Results Units/Value: <ul style="list-style-type: none"> Note: Deviations from protocol or guideline, analytical method. 	<p>Calculated and measured vapor pressure data for representative constituents of the C5 Non-Cyclics Category are listed below. The data identify a potential vapor pressure range for substances represented by the 15 CAS numbers under <u>Test Substance</u>. Substances in this category do not have a specific vapor pressure value. Actual vapor pressure ranges for substances in this category will vary dependent on their constituent composition.</p> <p>Commercial substances in this category consist of both high purity hydrocarbons and complex hydrocarbon reaction products with a carbon number distribution that is predominantly C5. The nine chemicals selected to represent the vapor pressure range of this category are C5 hydrocarbons that can be found in substances identified by the 15 CAS numbers.</p>

	<p>Constituents representing category members were selected on the basis of carbon number as identified by the category name, chemistry/structure, measured boiling point ranges for category substances, olefinic process (distillation) knowledge, and percentage of the composition of the represented process streams.</p> <table border="1" data-bbox="690 436 1372 808"> <thead> <tr> <th>Substance Constituent</th> <th>Calculated VP (hPa @ 25°C)</th> <th>Measured* VP (hPa @ 25°C)</th> </tr> </thead> <tbody> <tr> <td>cis-butene-2</td> <td>2.31 E³</td> <td>2.33 E³</td> </tr> <tr> <td>cis-pentene-2</td> <td>6.76 E²</td> <td>6.75 E²</td> </tr> <tr> <td>3-methyl-1-butene</td> <td>1.20 E³</td> <td>1.20 E³</td> </tr> <tr> <td>1,4-pentadiene</td> <td>9.79 E²</td> <td>9.97 E²</td> </tr> <tr> <td>Isopentane</td> <td>9.17 E²</td> <td>9.19 E²</td> </tr> <tr> <td>Isoprene</td> <td>7.35 E²</td> <td>7.33 E²</td> </tr> <tr> <td>n-pentane</td> <td>6.84 E²</td> <td>6.85 E²</td> </tr> <tr> <td>2-methyl-2-butene</td> <td>6.24 E²</td> <td>6.24 E²</td> </tr> <tr> <td>cyclopentene</td> <td>5.06 E²</td> <td>5.06 E²</td> </tr> </tbody> </table> <p>* Experimental values from EPIWIN database. The data represent a potential vapor pressure range for substances represented by the 15 CAS numbers under <u>Test Substance</u>.</p>	Substance Constituent	Calculated VP (hPa @ 25°C)	Measured* VP (hPa @ 25°C)	cis-butene-2	2.31 E ³	2.33 E ³	cis-pentene-2	6.76 E ²	6.75 E ²	3-methyl-1-butene	1.20 E ³	1.20 E ³	1,4-pentadiene	9.79 E ²	9.97 E ²	Isopentane	9.17 E ²	9.19 E ²	Isoprene	7.35 E ²	7.33 E ²	n-pentane	6.84 E ²	6.85 E ²	2-methyl-2-butene	6.24 E ²	6.24 E ²	cyclopentene	5.06 E ²	5.06 E ²
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	<p>The process streams in this category consist of high purity hydrocarbons or complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes and predominantly non-cyclic.</p> <p>More information on the C5 Non-Cyclics Category can be found in the American Chemistry Council, Olefins Panel test plan for this category (1).</p> <ol style="list-style-type: none"> Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.
Conclusion	<p>Based on calculated constituent data, substances in this category can have a vapor pressure range of 1.65 E³ to 3.45 E³ hPa @ 25°C. Based on measured constituent data, substances in this category can have a vapor pressure range of 1.68 E³ to 3.08 E³ hPa @ 25°C.</p>
Reliability	<p>(2) Reliable with restrictions</p> <p>The results include calculated data based on chemical structure as modeled by EPIWIN and measured data for specific chemicals as cited in the EPIWIN database. The data represent a potential vapor pressure range for substances represented by the 15 CAS numbers under <u>Test Substance</u>. This robust summary has a reliability rating of 2 because the data are not for specific substances in the C5 Non-Cyclics Category, but rather for selected constituents. These selected constituents represent all substances defined by this category and as such, this robust summary represents a "key study" for vapor pressure range based on constituent data.</p>
Reference	<p>EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA. (Vapor pressure values were calculated by the MPBPWIN subroutine and measured data came from the database in the computer program.)</p>

Vapor Pressure

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Test type GLP Year</p> <p><u>Results</u> Vapour pressure</p> <p><u>Remarks</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Naphthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was stable for the duration of the study.</p> <p>OECD Method 104 Vapour Pressure Yes 2002</p> <p>82260 Pa at 25°C (617 mm Hg)</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS/034. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Vapor Pressure

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Test type GLP Year</p> <p><u>Results</u> Vapour pressure</p> <p><u>Remarks</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s).</p> <p>Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8.</p> <p>The three major components analysed were shown to be stable for the duration of the study.</p> <p>OECD Method 104 Vapour Pressure Yes 2002</p> <p>58484 Pa at 25°C</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS035. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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	<u>Substance Constituent</u>	<u>Calculated WS (mg/L @ 25°C)</u>	<u>Measured WS* (mg/L @ 25°C)</u>
	cis-butene-2	652.7	423.5
	cis-pentene-2	245.1	na
	3-methyl-1-butene	242.7	na
	1,4-pentadiene	278.2	300.9
	Isopentane	184.6	na
	Isoprene	247.2	338.6
	n-pentane	159.7	49.8
	2-methyl-2-butene	218.7	206.1
	cyclopentene	307.2	na
	<p>* Experimental values from EPIWIN database. na = not available The data represent a potential water solubility range for substances represented by the 15 CAS numbers under <u>Test Substance</u>.</p>		
Test Substance	<p>The C5 Non-Cyclics Category includes the following CAS numbers:</p> <p>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 68476-55-1 Hydrocarbons, C5-rich 68477-35-0 Distillates, petroleum, C3-6, piperylene-rich 68514-39-6 Naphtha, petroleum, light steam-cracked, isoprene-rich 68527-11-7 Alkenes, C5 68527-19-5 Hydrocarbons, C1-4, 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 68606-29-1 Hydrocarbons, C4 and C8, butene concentrator by-product 68606-36-0 Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product 68956-55-8 Hydrocarbons, C5-unsatd. 78-79-5 1,3-Butadiene, 2-methyl-</p>		

	<p>C5 Non-Cyclics Category substances arise from production processes associated with ethylene manufacturing. The 15 CAS numbers are used to describe the ten process streams arising from the ethylene process and other associated C5 processes. The process streams in this category consist of high purity hydrocarbons or complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes and predominantly non-cyclic.</p> <p>More information on the C5 Non-Cyclics Category can be found in the American Chemistry Council, Olefins Panel test plan for this category (1).</p> <ol style="list-style-type: none"> Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.
Conclusion	<p>Based on calculated constituent data, substances in this category can have a water solubility range of 159.7 to 652.7 mg/L @ 25°C. Based on measured constituent data, substances in this category can have a water solubility range of 49.8 to 423.5 mg/L @ 25°C.</p>
Reliability	<p>(2) Reliable with restrictions</p> <p>The results include calculated data based on chemical structure as modeled by EPIWIN and measured data for specific chemicals as cited in the EPIWIN database. The data represent a potential water solubility range for substances represented by the 15 CAS numbers under <u>Test Substance</u>. This robust summary has a reliability rating of 2 because the data are not for specific substances in the C5 Non-Cyclics Category, but rather for selected constituents. These selected constituents represent all substances defined by this category and as such, this robust summary represents a "key study" for water solubility range based on constituent data.</p>
Reference	<p>EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA. (Water solubility values were calculated by the WSKOWWIN subroutine and measured data came from the database in the computer program.)</p>

ENVIRONMENTAL FATE ROBUST SUMMARIES**Photodegradation (Direct)**

Test Substance	Other TS [CAS # 513-35-9; 64742-83-2; 68410-97-9; 68476-43-7; 68476-55-1; 68477-35-0; 68514-39-6; 68527-11-7; 68527-19-5; 68603-00-9; 68603-03-2; 68606-29-1; 68606-36-0; 68956-55-8; 78-79-5]
Method/Guideline	Other: Technical discussion
Year (guideline)	Not applicable
GLP (Y/N)	Not applicable
Year (study performed)	Not applicable
Type (air, soil, water, other)	Water
Light Source	Not applicable
Light Spectrum	Not applicable
• Wave length value (upper/lower)	
Relative Intensity	Not applicable
Test Substance Spectrum	Not applicable
Test Conditions	Not applicable
• Note: Concentration, temperature, test system type, replication, deviations from guideline or protocol	
Direct Photolysis	<p><u>Summary</u></p> <p>In the environment, direct photolysis will not significantly contribute to the degradation of constituent chemicals in the C5 Non-Cyclics Category (C5 refers to a chemical with 5 carbons). The C5 Non-Cyclics Category includes ten process streams:</p> <ul style="list-style-type: none"> • Pyrolysis C5s • Hydrotreated C5s • Pentenes • Piperylene Concentrate • Isoprene Concentrate • Isoprene-Piperylene Concentrate • Isoprene, High Purity • 2-Methyl-2-Butene • Metathesis Byproduct <p>Fifteen CAS numbers (see <u>Test Substance</u>) identify substances</p>

	<p>derived from these process streams. As discussed below, the reaction process involved in direct photolysis occurs when sufficient light energy excites a molecule to the degree that a structural transformation occurs. In general, substances in this category do not contain component chemicals that will undergo direct photolysis.</p> <p><u>The C5 Non-Cyclics Category</u></p> <p>A process stream is a mixture of chemicals that arises from a chemical reaction or separation activity. The process streams in this category consist of both high purity hydrocarbons and complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes (with the exception of the Metathesis Byproduct stream which has 51% hexenes) and predominantly non-cyclic. All but two of these streams contain isoprene. The two streams without isoprene contain components such as 2-methyl-2-butene and pentenes, which are found in other streams in the category. That is why this group is considered a category for purposes of the High Production Volume (HPV) Chemical Program, and designated <u>C5 Non-Cyclics</u>.</p> <p>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 possible 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 or process).</p> <p>C5 Non-Cyclics streams arise from production processes associated with ethylene manufacturing. More information on the C5 Non-Cyclics category can be found in the American Chemistry Council, Olefins Panel test plan for this category (1). The plan is available on the U.S. Environmental Protection Agency website under the HPV Chemical Program. A brief description of the production and composition of the ten process streams in this category are:</p> <ul style="list-style-type: none"> • Pyrolysis C5s 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. • 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
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	<p>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.</p> <ul style="list-style-type: none"> • Pentenenes is the distillate that is sometimes removed during the fractionation of Pyrolysis C5s into concentrates of the reactive diolefins: isoprene, piperylene (1,3-pentadiene) and cyclopentadiene (as dimer). 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, Penetenenes can be removed later in the processing, for example by distillation of the Isoprene Concentrate. • Piperylene Concentrate is produced from Pyrolysis C5s by first "heat soaking" the stream in order to dimerize 1,3-cyclopentadiene (CPD). 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. • Isoprene Concentrate is also a distillate of the isoprene-piperylene splitter described above. The carbon number distribution is predominantly C5. A typical Isoprene Concentrate stream contains 40% isoprene with the balance largely iso- and n-pentane and C5 monoolefins. 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 pentene and pentane components in the Isoprene
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	<p>Concentrate.</p> <ul style="list-style-type: none"> • Isoprene-Piperylene Concentrate refers to the intermediate process stream charged to the isoprene-piperylene splitter (as described above for piperylene concentrate) and is sometimes isolated as a product. This stream typically contains about 20% isoprene and 14% piperylenes. • Isoprene, High Purity (98+%) is produced by separation from isoprene concentrate. This is accomplished using an extractive distillation process. • 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%. • 2-Methyl-2-Butene as a component 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. • Metathesis Byproduct refers to the byproduct that results from the Metathesis process, sometimes included in an olefins plant, which converts ethylene and/or butenes into propylene. The stream is a gasoline stream consisting primarily of C5 and C6 olefins. <p><u>Photolysis of Hydrocarbons</u></p> <p>The direct photolysis of an organic molecule occurs when it absorbs sufficient light energy to result in a structural transformation (2). The reaction process is initiated when light energy in a specific wavelength range elevates a molecule to an electronically excited state. However, the excited state is competitive with various deactivation processes that can result in the return of the molecule to a non excited state.</p> <p>The absorption of light in the ultra violet (UV)-visible range, 110-750 nm, can result in the electronic excitation of an organic molecule. Light in this range contains energy of the same order of magnitude as covalent bond dissociation energies (2). Higher wavelengths (e.g. infrared) result only in vibrational and rotational transitions, which do not tend to produce structural changes to a molecule.</p> <p>The stratospheric ozone layer prevents UV light of less than 290 nm from reaching the earth's surface. Therefore, only light at wavelengths between 290 and 750 nm can result in photochemical transformations in the environment (2). Although the absorption of UV light in the 290-750 nm range is</p>
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	<p>necessary, it is not always sufficient for a chemical to undergo photochemical degradation. Energy may be re-emitted from an excited molecule by mechanisms other than chemical transformation, resulting in no change to the parent molecule.</p> <p>A conservative approach to estimating a photochemical degradation rate is to assume that degradation will occur in proportion to the amount of light wavelengths >290 nm absorbed by the molecule (3). Saturated hydrocarbons do not absorb light above 200 nm. Some characteristic absorbance maxima (λ_{\max}) and associated molar absorptivities (ϵ) for selected unsaturated hydrocarbons are shown below (2):</p> <table border="1" data-bbox="690 577 1385 745"> <thead> <tr> <th rowspan="2"><u>Hydrocarbon</u></th> <th colspan="2">I below 290 nm</th> <th>I above 290 nm</th> </tr> <tr> <th>λ_{\max}</th> <th>ϵ</th> <th>λ_{\max}</th> </tr> </thead> <tbody> <tr> <td>Ethylene</td> <td>193</td> <td>10,000</td> <td>-</td> </tr> <tr> <td>1,3-Butadiene</td> <td>217</td> <td>2,090</td> <td>-</td> </tr> <tr> <td>Benzene</td> <td>255</td> <td>215</td> <td>-</td> </tr> </tbody> </table> <p>Olefins with one double bond, or two conjugated double bonds, which constitute the majority of the chemicals in the C5 Non-Cyclics Category, do not absorb appreciable light energy above 290 nm. The absorption of UV light to cause cis-trans isomerism about the double bond of an olefin occurs only if it is in conjugation with an aromatic ring (2).</p> <p>Substances in the C5 Non-Cyclics Category do not contain component molecules that will undergo direct photolysis. Therefore, this fate process will not contribute to a measurable degradative removal of chemical components in this category from the environment.</p> <p>References</p> <ol style="list-style-type: none"> Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. Virginia, USA. Harris, J. C. 1982. "Rate of Aqueous Photolysis," Chapter 8 in: W. J. Lyman, W. F. Reehl, and D. H. Rosenblatt, eds., Handbook of Chemical Property Estimation Methods, McGraw-Hill Book Company, New York, USA. Zepp, R. G. and D. M. Cline. 1977. Rates of Direct Photolysis in the Aqueous Environment, Environ. Sci. Technol., 11:359-366. 	<u>Hydrocarbon</u>	I below 290 nm		I above 290 nm	λ_{\max}	ϵ	λ_{\max}	Ethylene	193	10,000	-	1,3-Butadiene	217	2,090	-	Benzene	255	215	-
<u>Hydrocarbon</u>	I below 290 nm		I above 290 nm																	
	λ_{\max}	ϵ	λ_{\max}																	
Ethylene	193	10,000	-																	
1,3-Butadiene	217	2,090	-																	
Benzene	255	215	-																	
<p>Indirect Photolysis</p> <ul style="list-style-type: none"> Results: type of sensitizer, concentration of sensitizer, rate constant, % degradation, half-life 	Not applicable																			
<p>Degradation Products</p> <ul style="list-style-type: none"> Note: Identification, concentration 	Unknown																			

Test Substance	<p>The C5 Non-Cyclics Category includes the following CAS numbers:</p> <p>513-35-9 2-Butene, 2-methyl-</p> <p>64742-83-2 Naphtha, petroleum, light steam-cracked</p> <p>68410-97-9 Distillates, petroleum, light distillate hydrotreating process, low-boiling</p> <p>68476-43-7 Hydrocarbons, C4-6, C5-rich</p> <p>68476-55-1 Hydrocarbons, C5-rich</p> <p>68477-35-0 Distillates, petroleum, C3-6, piperylene-rich</p> <p>68514-39-6 Naphtha, petroleum, light steam-cracked, isoprene-rich</p> <p>68527-11-7 Alkenes, C5</p> <p>68527-19-5 Hydrocarbons, C1-4, debutanizer fraction</p> <p>68603-00-9 Distillates, petroleum, thermal cracked naphtha and gas oil</p> <p>68603-03-2 Distillates, petroleum, thermal cracked naphtha and gas oil, extractive</p> <p>68606-29-1 Hydrocarbons, C4 and C8, butene concentrator by-product</p> <p>68606-36-0 Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product</p> <p>68956-55-8 Hydrocarbons, C5-unsatd.</p> <p>78-79-5 1,3-Butadiene, 2-methyl-</p>
Conclusion	Not applicable
Reliability	These data represent a key study for characterizing the potential of substances in the C5 Non-Cyclics Category to undergo direct photodegradation.
Reference	American Chemistry Council, Olefins Panel. 2002. Photodegradation (Direct): C5 Non-Cyclics Category. Rosslyn, VA, USA.

Photodegradation (Indirect)

Test Substance	Other TS [CAS # 513-35-9; 64742-83-2; 68410-97-9; 68476-43-7; 68476-55-1; 68477-35-0; 68514-39-6; 68527-11-7; 68527-19-5; 68603-00-9; 68603-03-2; 68606-29-1; 68606-36-0; 68956-55-8; 78-79-5]
Method/Guideline	Calculated values using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04
Year (guideline)	1999
GLP (Y/N)	Not applicable
Year (study performed)	Not applicable
Type (air, soil, water, other)	Not applicable
Light Source	Sunlight
Light Spectrum • Wave length value (upper/lower)	Natural sunlight
Relative Intensity	1
Test Substance Spectrum	Not applicable
Test Conditions • Note: Concentration, temperature, test system type, replication, deviations from guideline or protocol	Indirect photodegradation, or atmospheric oxidation potential, is based on the structure-activity relationship methods developed by R. Atkinson. Temperature: 25°C Sensitizer: OH radical Concentration of Sensitizer: $1.5 \text{ E}^6 \text{ OH radicals/cm}^3$
Direct Photolysis Results: half-life, % degradation, quantum yield	Not applicable

<p>Indirect Photolysis</p> <ul style="list-style-type: none">Results: type of sensitizer, concentration of sensitizer, rate constant, % degradation, half-life	<p><u>The C5 Non-Cyclics Category</u></p> <p>C5 Non-Cyclics Category substances arise from production processes associated with ethylene manufacturing. The 15 CAS numbers are used to describe the ten process streams arising from the ethylene process and other associated C5 processes.</p> <p>Commercial products in this category consist of both high purity hydrocarbons and complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes and predominantly non-cyclic. That is why this group is considered a category for purposes of the High Production Volume (HPV) Chemical Program, and designated <u>C5 Non-Cyclics</u>.</p> <p>The nine chemicals selected to represent the atmospheric oxidation potential of this category are C5 hydrocarbons that can be found in substances identified by the 15 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category name, chemistry/structure, measured boiling point ranges for category substances, olefinic process (distillation) knowledge, and percentage of the composition of the represented process streams.</p> <p><u>Atmospheric Oxidation of Hydrocarbons</u></p> <p>In the environment, organic chemicals emitted into the troposphere are degraded by several important transformation processes. The dominant transformation process for most compounds is the daylight reaction with hydroxyl (OH-) radicals (Atkinson, 1988, 1989). The rate at which an organic compound reacts with OH- radicals is a direct measure of its atmospheric persistence (Meylan and Howard, 1993).</p> <p>AOPWIN estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radicals.</p> <p>Since the reactions only take place in the presence of sunlight, the atmospheric half-lives are normalized for a 12-hour day.</p>
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	<u>Chemical</u>	Calculated* <u>half-life (hrs)</u>	OH- Rate Constant <u>(cm³/molecule-sec)</u>
	cis-butene-2	2.3	56.7 E ⁻¹²
	cis-pentene-2	2.2	57.6 E ⁻¹²
	3-methyl-1-butene	4.5	28.6 E ⁻¹²
	1,4-pentadiene	2.4	53.5 E ⁻¹²
	Isopentane	31.8	4.0 E ⁻¹²
	Isoprene	1.2	105.1 E ⁻¹²
	n-pentane	31.7	4.0 E ⁻¹²
	2-methyl-2-butene	1.5	87.3 E ⁻¹²
	cyclopentene	2.2	58.8 E ⁻¹²
	* Atmospheric half-life values are based on a 12-hr day.		
	More information on the C5 Non-Cyclics Category can be found in the American Chemistry Council, Olefins Panel test plan for this category (Olefins Panel, 2001).		
	<u>References:</u>		
	1. Atkinson, R. 1988. Estimation of gas-phase hydroxyl radical rate constants for organic chemicals. <i>Environ. Toxicol. Chem.</i> 7:435-442.		
	2. Atkinson, R. 1989. Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds. J. Phys. Chem. Ref. Data Monograph No. 1, Amer. Inst. Physics & Amer. Chem. Soc., NY.		
	3. Meylan, W.M. and P.H. Howard. 1993. Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone. <i>Chemosphere</i> 12:2293-2299.		
	4. Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.		
Degradation Products	Unknown		
<ul style="list-style-type: none"> Note: Identification, concentration 			

Test Substance	<p>The C5 Non-Cyclics Category includes the following CAS numbers:</p> <p>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 68476-55-1 Hydrocarbons, C5-rich 68477-35-0 Distillates, petroleum, C3-6, piperylene-rich 68514-39-6 Naphtha, petroleum, light steam-cracked, isoprene-rich 68527-11-7 Alkenes, C5 68527-19-5 Hydrocarbons, C1-4, 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 68606-29-1 Hydrocarbons, C4 and C8, butene concentrator by-product 68606-36-0 Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product 68956-55-8 Hydrocarbons, C5-unsatd. 78-79-5 1,3-Butadiene, 2-methyl-</p>
Conclusion	<p>Atmospheric oxidation via hydroxyl radicals can be a significant route of degradation for products in this category. Based on calculated values, products in this category can have an atmospheric half-life range of 1.2 to 31.8 hours as a result of indirect photolysis by hydroxyl radical attack.</p>
Reliability	<p>(2) Reliable with restrictions</p> <p>The results include calculated data based on chemical structure as modeled by AOPWIN. The data represent a potential atmospheric half-life range for substances represented by the 15 CAS numbers under <u>Test Substance</u>. This robust summary has a reliability rating of 2 because the data are not for specific substances in the C5 Non-Cyclics Category, but rather for selected constituents. These selected constituents represent all substances defined by this category and as such, this robust summary represents a "key study" for atmospheric half-life range based on constituent data.</p>
Reference	<p>Meylan, M., SRC 1994-1999. AOPWIN is contained in the computer program EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.</p>

Hydrolysis (Stability in Water)

Test Substance	Other TS [CAS # 513-35-9; 64742-83-2; 68410-97-9; 68476-43-7; 68476-55-1; 68477-35-0; 68514-39-6; 68527-11-7; 68527-19-5; 68603-00-9; 68603-03-2; 68606-29-1; 68606-36-0; 68956-55-8; 78-79-5]
Method/Guideline	Other: Technical discussion
Year (guideline)	Not applicable
Type (test type)	Not applicable
GLP (Y/N)	Not applicable
Year (study performed)	Not applicable
Analytical Monitoring	Not applicable
Test Conditions • Note: Concentration preparation, vessel type, volume, replication, deviations from guideline or protocol	Not applicable
Results Units/Value: • Note: Analytical method, observations, half-lives by pH, degradation products	Not applicable
Test Substance	The C5 Non-Cyclics Category includes the following CAS numbers: 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 68476-55-1 Hydrocarbons, C5-rich 68477-35-0 Distillates, petroleum, C3-6, piperylene-rich 68514-39-6 Naphtha, petroleum, light steam-cracked, isoprene-rich 68527-11-7 Alkenes, C5 68527-19-5 Hydrocarbons, C1-4, 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 68606-29-1 Hydrocarbons, C4 and C8, butene concentrator by-product 68606-36-0 Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product

	<p>68956-55-8 Hydrocarbons, C5-unsatd. 78-79-5 1,3-Butadiene, 2-methyl-</p> <p>C5 Non-Cyclics Category substances arise from production processes associated with ethylene manufacturing. The 15 CAS numbers are used to describe the ten process streams arising from the ethylene process and other associated C5 processes. The process streams in this category consist of high purity hydrocarbons or complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes and predominantly non-cyclic.</p> <p>More information on the C5 Non-Cyclics Category can be found in the American Chemistry Council, Olefins Panel test plan for this category (1).</p> <p>1. Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.</p>
<p>Conclusion</p>	<p><u>Summary</u></p> <p>In the environment, hydrolysis will not contribute to the degradation of chemicals in the C5 Non-Cyclics Category (C5 refers to a chemical with 5 carbons). The C5 Non-Cyclics Category includes ten process streams:</p> <ul style="list-style-type: none"> • Pyrolysis C5s • Hydrotreated C5s • Pentenes • Piperylene Concentrate • Isoprene Concentrate • Isoprene-Piperylene Concentrate • Isoprene, High Purity • 2-Methyl-2-Butene • Metathesis Byproduct <p>Fifteen CAS numbers (see <u>Test Substance</u>) identify substances derived from these process streams. As discussed below, the chemicals in these streams are composed of carbon and hydrogen and are not amenable to hydrolysis because of their molecular structure and the chemical reaction required for this type of transformation to occur.</p> <p><u>The C5 Non-Cyclics Category</u></p> <p>A process stream is a mixture of chemicals that arises from a chemical reaction or separation activity. The process streams in this category consist of both high purity hydrocarbons and complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes (with the exception of the Metathesis Byproduct stream which has 51% hexenes) and predominantly</p>

	<p>non-cyclic. All but two of these streams contain isoprene. The two streams without isoprene contain components such as 2-methyl-2-butene and pentenes, which are found in other streams in the category. That is why this group is considered a category for purposes of the High Production Volume (HPV) Chemical Program, and designated <u>C5 Non-Cyclics</u>.</p> <p>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 possible 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 or process).</p> <p>C5 Non-Cyclics streams arise from production processes associated with ethylene manufacturing. More information on the C5 Non-Cyclics Category can be found in the American Chemistry Council, Olefins Panel test plan for this category (1). The plan is available on the U.S. Environmental Protection Agency website under the HPV Chemical Program. A brief description of the production and composition of the ten process streams in this category are:</p> <ul style="list-style-type: none">• Pyrolysis C5s 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.• 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
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	<p>more volatile C6 hydrocarbons. Benzene content is typically 1%. Unlike pyrolysis C5s, the diolefin content in Hydrotreated C5s is very low.</p> <ul style="list-style-type: none"> • Pentenenes is the distillate that is sometimes removed during the fractionation of Pyrolysis C5s into concentrates of the reactive diolefins: isoprene, piperylene (1,3-pentadiene) and cyclopentadiene (as dimer). 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 the processing, for example by distillation of the Isoprene Concentrate. • Piperylene Concentrate is produced from Pyrolysis C5s by first "heat soaking" the stream in order to dimerize 1,3-cyclopentadiene (CPD). 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. • Isoprene Concentrate is also a distillate of the isoprene-piperylene splitter described above. The carbon number distribution is predominantly C5. A typical Isoprene Concentrate stream contains 40% isoprene with the balance largely iso- and n-pentane and C5 monoolefins. 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 pentene and pentane components in the Isoprene Concentrate. • Isoprene-Piperylene Concentrate refers to the intermediate process stream charged to the isoprene-piperylene splitter (as described above for piperylene concentrate) and is sometimes isolated as a product. This stream typically contains about 20% isoprene and 14% piperylenes. • Isoprene, High Purity (98+%) is produced by separation from isoprene concentrate. This is accomplished using an extractive distillation process. • Isoprene Purification Byproduct is a byproduct from
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	<p>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%.</p> <ul style="list-style-type: none"> • 2-Methyl-2-Butene as a component 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. • Metathesis Byproduct refers to the byproduct that results from the Metathesis process, sometimes included in an olefins plant, which converts ethylene and/or butenes into propylene. The stream is a gasoline stream consisting primarily of C5 and C6 olefins. <p><u>Hydrolysis of Hydrocarbons as a Function of Molecular Structure</u></p> <p>Hydrolysis of an organic molecule occurs when a molecule (R-X) reacts with water (H₂O) to form a new carbon-oxygen bond after the carbon-X bond is cleaved (2,3). Mechanistically, this reaction is referred to as a nucleophilic substitution reaction, where X is the leaving group being replaced by the incoming nucleophilic oxygen from the water molecule.</p> <p>The leaving group, X, must be a molecule other than carbon because for hydrolysis to occur, the R-X bond cannot be a carbon-carbon bond. The carbon atom lacks sufficient electronegativity to be a good leaving group and carbon-carbon bonds are too stable (high bond energy) to be cleaved by nucleophilic substitution. Thus, hydrocarbons, including alkenes, are not subject to hydrolysis (3) and this fate process will not contribute to the degradative loss of chemical components in this category from the environment.</p> <p>Under strongly acidic conditions the carbon-carbon double bond found in alkenes, such as those in the C5 Non-Cyclics Category, will react with water by an addition reaction mechanism (2). The reaction product is an alcohol. This reaction is not considered to be hydrolysis because the carbon-carbon linkage is not cleaved and because the reaction is freely reversible (3). Substances that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (4).</p> <p>The substances in the C5 Non-Cyclics Category are primarily olefins that contain at least one double bond (alkenes). The remaining chemicals are saturated hydrocarbons (alkanes). These two groups of chemicals contain only carbon and hydrogen. As such, their molecular structure is not subject to the hydrolytic mechanism discussed above. Therefore, chemicals in the C5 Non-Cyclics Category have a very low potential to hydrolyze, and this degradative process will not</p>
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	<p>contribute to their removal in the environment.</p> <p>References</p> <ol style="list-style-type: none"> 1. Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA. 2. Gould, E.S. (1959), Mechanism and Structure in Organic Chemistry, Holt, Reinhart and Winston, New York, NY, USA. 3. Harris, J.C. (1982), "Rate of Hydrolysis," Chapter 7 in: W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt, eds., Handbook of Chemical Property Estimation Methods, McGraw-Hill Book Company, New York, NY, USA. 4. Neely, W. B. 1985. Hydrolysis. In: W. B. Neely and G. E. Blau, eds. Environmental Exposure from Chemicals. Vol I, pp. 157-173. CRC Press, Boca Raton, FL, USA.
Reliability	These data represent a key study for characterizing the potential of substances in the C5 Non-Cyclics Category to undergo hydrolysis.
Reference	American Chemistry Council, Olefins Panel. 2002. Hydrolysis: C5 Non-Cyclics Category. Rosslyn, VA, USA.

Transport / Distribution (Fugacity)

Test Substance	Other TS [CAS # 513-35-9; 64742-83-2; 68410-97-9; 68476-43-7; 68476-55-1; 68477-35-0; 68514-39-6; 68527-11-7; 68527-19-5; 68603-00-9; 68603-03-2; 68606-29-1; 68606-36-0; 68956-55-8; 78-79-5]
Method/Guideline	Calculated according to Mackay Level I, EQC Model version 1.01
Year (guideline)	1997
Type (test type)	Not applicable
GLP	Not applicable
Year (study performed)	Not applicable
Estimation Temperature	25°C
Test Conditions <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, test conditions. 	<p>The EQC Level I is a steady state, equilibrium model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment.</p> <p>Physicochemical input values for the model were calculated using the EPIWIN Estimation v 3.04 program (1). Measured input values were also used where available and obtained from the EPIWIN database (1). Distribution data from the equilibrium model provide basic information on the potential partitioning behavior of chemicals between selected environmental compartments (i.e., air, water, soil, sediment, suspended sediment, biota).</p> <p>1. EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.</p>

<p>Results</p> <p>Units/Value:</p> <ul style="list-style-type: none"> Note: Deviations from protocol or guideline, analytical method. 	<p>Calculated partitioning data for representative constituents of the C5 Non-Cyclics Category are listed below. The data identify a potential distribution for substances represented by the 15 CAS numbers under <u>Test Substance</u>. Actual distribution of substances in this category will vary dependent on their constituent composition.</p> <p>Commercial substances in this category consist of both high purity hydrocarbons and complex hydrocarbon reaction products with a carbon number distribution that is predominantly C5. The nine chemicals selected to represent the environmental distribution range of this category are C5 hydrocarbons that are common across the 15 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category name, chemistry/structure, measured boiling point ranges for category substances, and olefinic process (distillation) knowledge.</p> <p>The range of distribution data for constituent chemicals in each of the compartments can be used as an estimate of the partitioning behavior for category substances.</p> <p>The following Mackay Level I model distribution values for representative constituents of substances in this category were determined using physicochemical input data calculated using the EPIWIN program:</p> <table border="1" data-bbox="685 1003 1385 1402"> <thead> <tr> <th rowspan="3"><u>Chemical</u></th> <th colspan="2"><u>Calculated*</u></th> <th colspan="2"><u>Measured**</u></th> </tr> <tr> <th colspan="2"><u>Percent Distribution</u></th> <th colspan="2"><u>Percent Distribution</u></th> </tr> <tr> <th><u>Air</u></th> <th><u>Water</u></th> <th><u>Air</u></th> <th><u>Water</u></th> </tr> </thead> <tbody> <tr> <td>cis-butene-2</td> <td>99.97</td> <td>0.03</td> <td>99.98</td> <td>0.02</td> </tr> <tr> <td>cis-pentene-2</td> <td>99.97</td> <td>0.03</td> <td>99.97</td> <td>0.03</td> </tr> <tr> <td>3-methyl-1-butene</td> <td>99.98</td> <td>0.02</td> <td>99.98</td> <td>0.02</td> </tr> <tr> <td>1,4-pentadiene</td> <td>99.97</td> <td>0.02</td> <td>99.97</td> <td>0.02</td> </tr> <tr> <td>Isopentane</td> <td>99.98</td> <td>0.01</td> <td>99.98</td> <td>0.01</td> </tr> <tr> <td>Isoprene</td> <td>99.97</td> <td>0.02</td> <td>99.96</td> <td>0.03</td> </tr> <tr> <td>n-pentane</td> <td>99.97</td> <td>0.02</td> <td>99.99</td> <td>0.01</td> </tr> <tr> <td>2-methyl-2-butene</td> <td>99.97</td> <td>0.03</td> <td>99.97</td> <td>0.02</td> </tr> <tr> <td>cyclopentene</td> <td>99.94</td> <td>0.04</td> <td>99.94</td> <td>0.04</td> </tr> </tbody> </table> <p>* Distribution values determined using calculated input data from EPIWIN program</p> <p>** Distribution values determined using input data from the EPIWIN program experimental database</p> <p>Distribution of each chemical to each remaining compartment (soil, sediment, suspended sediment, biota) was calculated as 0.01% or less. Mobility in the environment is expected to be high due to the relatively high water solubility and high vapor pressure of these chemicals.</p>	<u>Chemical</u>	<u>Calculated*</u>		<u>Measured**</u>		<u>Percent Distribution</u>		<u>Percent Distribution</u>		<u>Air</u>	<u>Water</u>	<u>Air</u>	<u>Water</u>	cis-butene-2	99.97	0.03	99.98	0.02	cis-pentene-2	99.97	0.03	99.97	0.03	3-methyl-1-butene	99.98	0.02	99.98	0.02	1,4-pentadiene	99.97	0.02	99.97	0.02	Isopentane	99.98	0.01	99.98	0.01	Isoprene	99.97	0.02	99.96	0.03	n-pentane	99.97	0.02	99.99	0.01	2-methyl-2-butene	99.97	0.03	99.97	0.02	cyclopentene	99.94	0.04	99.94	0.04
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Test Substance	<p>The C5 Non-Cyclics Category includes the following CAS numbers:</p> <p>513-35-9 2-Butene, 2-methyl-</p> <p>64742-83-2 Naphtha, petroleum, light steam-cracked</p> <p>68410-97-9 Distillates, petroleum, light distillate hydrotreating process, low-boiling</p> <p>68476-43-7 Hydrocarbons, C4-6, C5-rich</p> <p>68476-55-1 Hydrocarbons, C5-rich</p> <p>68477-35-0 Distillates, petroleum, C3-6, piperylene-rich</p> <p>68514-39-6 Naphtha, petroleum, light steam-cracked, isoprene-rich</p> <p>68527-11-7 Alkenes, C5</p> <p>68527-19-5 Hydrocarbons, C1-4, debutanizer fraction</p> <p>68603-00-9 Distillates, petroleum, thermal cracked naphtha and gas oil</p> <p>68603-03-2 Distillates, petroleum, thermal cracked naphtha and gas oil, extractive</p> <p>68606-29-1 Hydrocarbons, C4 and C8, butene concentrator by-product</p> <p>68606-36-0 Hydrocarbons, C5-unsatd. rich, isoprene purifn. by-product</p> <p>68956-55-8 Hydrocarbons, C5-unsatd.</p> <p>78-79-5 1,3-Butadiene, 2-methyl-</p>
	<p>C5 Non-Cyclics Category substances arise from production processes associated with ethylene manufacturing. The 15 CAS numbers are used to describe the ten process streams arising from the ethylene process and other associated C5 processes. The process streams in this category consist of high purity hydrocarbons or complex hydrocarbon reaction products that are predominantly C5 alkanes or alkenes and predominantly non-cyclic.</p>
	<p>More information on the C5 Non-Cyclics Category can be found in the American Chemistry Council, Olefins Panel test plan for this category (1).</p>
	<p>1. Olefins Panel, HPV Implementation Task Group. 2001. High Production Volume (HPV) Chemical Challenge Program Test Plan For The C5 Non-Cyclics Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.</p>

Conclusion	<p>The partitioning data represent a potential distribution range for substances in the 15 CAS numbers listed under <u>Test Substance</u>. Substances in the C5 Non-Cyclics Category are calculated to partition primarily to air with a smaller percentage partitioning to water. Relatively high vapor pressure and high water solubility largely control the partitioning behavior of constituent chemicals in substances from this category.</p> <p>The input data used to run the EQC Level I model included estimated values calculated by the EPIWIN program based on chemical structure and measured data from the EPIWIN database. A comparison of the distribution data developed using either all calculated input values or measured values where data were available indicate a similar partitioning behavior and support the use of the dataset for chemicals without any measured data.</p>
Reliability	<p>(2) Reliable with restrictions</p> <p>The input data used to run the EQC Level I model include calculated and experimental values available through the EPIWIN program. The data represent a potential environmental distribution range for substances with the 15 CAS numbers listed under <u>Test Substance</u>. This robust summary has a reliability rating of 2 because the data are not for specific substances in the C5 Non-Cyclics Category, but rather for selected constituents. These selected constituents represent all substances defined by this category and as such, this robust summary represents a "key study" for distribution range based on constituent data.</p>
Reference	<p>Mackay, D.A. DiGuardo, S. Paterson, and C. Cowan. EQC Model Version 1.01. 1997. Available from the Environmental Modeling Centre, Trent University, Canada.</p>

Biodegradation

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed</p> <p>GLP Year Type: Inoculum: Concentration: Contact time:</p> <p>Test inoculum preparation</p> <p>Study design</p> <p><u>Results</u></p>	<p>Isoprene (CAS No. 78-79-5) also known as 1,3-butadiene,2-methyl</p> <p>The test substance was stable for the duration of all studies performed by the test house.</p> <p>The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (88%) was equivalent to 102% of the theoretical value (85.9%).</p> <p>OECD Guide-line 301D and EC Directive 92/69, C.4-E and EPA OPPTS 835.3110;</p> <p>Yes 2002 Aerobic Domestic sewage effluent 2mg/l related to test substance 28 days</p> <p>Main test A sample of secondary effluent was collected on the day of the test from a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).</p> <p>Main test Eighteen bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1ml/l, and the test substance at a nominal loading of 2 mg/l. The dissolved oxygen (DO) concentration in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. Four bottles were established for a concurrent five-day microbial inhibition assay, in which the biodegradation of the readily biodegradable reference substance, sodium benzoate, was examined in the presence of the test substance. DO concentration was measured in replicate bottles on Days 0 and 5.</p> <p>A further eighteen bottles were filled with mineral salts, inoculated with unacclimated sewage effluent at a loading of 1 ml/l, and the reference substance, sodium benzoate at a nominal loading of 5 mg/l. DO concentrations in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. All test systems were incubated at 22 +/- 2°C in darkness. Theoretical oxygen demands for the test and reference substances were based on their empirical formulae and molecular weights. The study was initiated on 13 March 2002. A maximum of 60% biodegradation was measured on Day 28 of the main Closed Bottle test. Biodegradation was not established in five BOD bottles on or after Day 18 of the test, but had occurred (54%) in the three remaining bottles measured</p>
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Study design	<p>on or after Day 18.</p> <p>The mean Total Viable Count of the sample of final sewage effluent in the main test was 7.1×10^5 Colony forming Units (CFU) per ml and the mean count in inoculated MSM on Day 0 was 3.3×10^4 cfu/ml.</p> <p>The presence of the test substance did not cause any inhibitory affect on the normal degradative activity of the microbial inoculum on the reference substance.</p>
	<p>Supplementary investigation</p> <p>Eight bottles were established with mineral salts medium and an inoculum of medium (10 ml/l) from a BOD bottle incubated for 28 days as part of the main test (isoprene degradation in the BOD bottle used had achieved 60% on Day 28). Four bottles were of a modified design and were treated with isoprene (nominally 2 mg/l). Four bottles contained inoculum and mineral salts medium alone.</p> <p>The DO, pH and temperature of two bottles containing isoprene and two containing inoculum and mineral salts medium alone were analysed on Day 0 and following seven days of incubation in darkness at $22 \pm 2^\circ\text{C}$.</p> <p>A maximum of 64% biodegradation was measured on Day 7 of the supplementary investigation. This confirmed that isoprene was biodegradable in the presence of a pre-exposed inoculum.</p>
Degradation Kinetic of test substance	<p>60 (\pm) % after 28 day(s)</p> <p>5 day(s) 5 and 2 %</p> <p>7 day(s) 4 and 4 %</p> <p>11 day(s) 3 and 2 %</p> <p>14 day(s) 5 and 5 %</p> <p>18 day(s) 60 and 2 %</p> <p>21 day(s) 7 and 54 %</p> <p>25 day(s) 9 and 13%</p> <p>28 day(s) 58 and 2 %</p>
Control substance Kinetic	<p>Benzoic acid, sodium salt</p> <p>5 day(s) 79 and 78 %</p> <p>28 day(s) 86 and 86 %</p>
<u>Conclusions</u>	Biodegradable in the presence of pre-exposed inoculum
<u>Data Quality</u>	(1) Valid without restriction
<u>Reference</u>	<p>Huntingdon Life Sciences Ltd.2003. Assessment of biodegradability using the closed bottle method Project ID CSS 036. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>

Biodegradation

Test Substance	Isoprene, inhibited with 100 ppm p-tert-butylcatechol CAS No. 78-79-5: CAS Inventory Name: 1,3-Butadiene, 2-methyl-
Method/Guideline	OECD Guideline 301F
Year (guideline)	1992
Type (test type)	Ready Biodegradability: Manometric Respirometry Test
GLP (Y/N)	Yes
Year (study performed)	2003
Inoculum	Domestic activated sludge
Exposure Period	28 Days
Test Conditions	<p>Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 51mg/L and 46 mg/L, respectively. Blank test systems, which did not contain the test or positive control substance, were run concurrently in triplicate.</p> <p>The total suspended solids (TSS) of the activated sludge was determined to be 4.28 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The microbial count of the inoculum was 10⁵ CFU/mL. One liter of test medium, which was aerated for 24 hours with carbon dioxide free air, was added to each one liter respirometer flask. The test substance was weighed in an air tight syringe and injected into the test medium. The test system was sealed immediately after addition of the test substance. An aliquot of the positive control stock solution was added to the appropriate test flasks.</p> <p>An unacclimated activated sludge inoculum was used in this study. The inoculum was obtained from the Clinton Sanitary Wastewater Treatment Plant, Annandale, NJ, USA. The treatment plant receives domestic sewage.</p> <p>All test systems were placed on a Coordinated Environmental Services (CES) automated respirometer which automatically recorded the oxygen uptake in general agreement with the OECD guideline. The 28-day study was conducted at a temperature range of 22 ± 1°C.</p>
<ul style="list-style-type: none"> Note: Concentration preparation, vessel type, replication, test conditions. 	

<p>Results</p> <p>Units/Value</p> <p>Note: Deviations from protocol or guideline analytical method.</p>	<p>Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test substance as calculated using results of an elemental analysis of the test substance.</p> <p>By day 14, >60% biodegradation of positive control was observed, which meets the guideline requirement. No deviations from the protocol occurred that affected the integrity of the study data.</p> <p>The average percent biodegradation of the test substance was determined to be 61% on day 28. The test substance can be considered readily biodegradable.</p> <table border="1" data-bbox="690 646 1299 808"> <thead> <tr> <th></th> <th>% Degradation*</th> <th>Mean %</th> </tr> </thead> <tbody> <tr> <td>Degradation</td> <td></td> <td></td> </tr> <tr> <td><u>Sample</u></td> <td><u>(day 28)</u></td> <td><u>(day 28)</u></td> </tr> <tr> <td>Test Substance</td> <td>75, 55, 53</td> <td>61</td> </tr> <tr> <td>Na Benzoate</td> <td>91, 100, 89</td> <td>94</td> </tr> </tbody> </table> <p>* replicate data</p>		% Degradation*	Mean %	Degradation			<u>Sample</u>	<u>(day 28)</u>	<u>(day 28)</u>	Test Substance	75, 55, 53	61	Na Benzoate	91, 100, 89	94
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<p>Conclusion</p>	<p>Readily biodegradable</p>															
<p>Reliability</p>	<p>(1) Reliable without restriction.</p>															
<p>Reference</p>	<p>ExxonMobil Biomedical Sciences, Inc. 2004. Ready Biodegradability: Manometric Respirometry test. Study # 177294A.</p>															

Biodegradation

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed</p> <p>GLP Year Type Inoculum Concentration Contact time Test inoculum preparation</p> <p>Study design</p>	<p>2-methyl-2-butene (CAS No. 513-35-9) The test substance was stable for the duration of all studies performed at the test house.</p> <p>The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (85.47%) was equivalent to 99.8% of the theoretical value (85.63%), which was calculated using the empirical formula of 2-methyl-2-butene which comprised 98.2% of the test substance.</p> <p>OECD Guideline 301D and EC Directive 92/69, C.4-E and EPA OPPTS 835.3110 Yes 2001-2002 Aerobic Domestic sewage effluent 2.1 mg/l related to test substance 28 days A sample of secondary effluent was collected on the day of the test from a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).</p> <p>Eighteen bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/l, and the test substance at a nominal loading of 2.1 mg/l. The dissolved oxygen (DO) concentration in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. Four bottles were established for a concurrent five-day microbial inhibition assay, in which the biodegradation of the readily biodegradable reference substance, sodium benzoate, was examined in the presence of the test substance. DO concentration was measured in replicate bottles on Days 0 and 5. A further eighteen bottles were filled with mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/l, and the reference substance, sodium benzoate at a nominal loading of 5 mg/l. DO concentrations in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. All test systems were incubated at $22 \pm 2^\circ\text{C}$ in darkness. Theoretical oxygen demands for the test and reference substances were based on their empirical formulae and molecular weights. The study was initiated on 8 October 2001.</p>
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<p><u>Results</u></p> <p>Degradation</p> <p>Kinetic of test substance</p> <p>Control substance</p> <p>Kinetic</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>A maximum of 15% biodegradation was measured (on Day 25) by the end of the Closed Bottle test.</p> <p>The mean Total Viable Count of the sample of final sewage effluent in the main test was 1.0×10^5 Colony Forming Units (CFU) per ml and the mean count in inoculated MSM on Day 0 was 8.2×10^3 CFU/ml.</p> <p>The presence of the test substance was not considered to have caused any inhibitory affect on the normal degradative activity of the microbial inoculum on the reference substance in one replicate mixture containing both substances. There was discolouration of the contents of the second bottle of the inhibition assay, the pH was lower and there was no evidence of sodium benzoate degradation. Therefore, this result was considered to be erroneous.</p> <p>7 (\pm) % after 28 day(s)</p> <p>5 day(s) 2 and 4% 7 day(s) 1 and 2% 11 day(s) 0 and 1% 14 day(s) 2 and 2% 18 day(s) 4 and 4% 21 day(s) 2 and 6% 25 day(s) 12 and 15% 28 day(s) 4 and 10%</p> <p>Benzoic acid, sodium salt</p> <p>5 day(s) 67 and 68% 28 day(s) 83 and 85%</p> <p>Not biodegradable</p> <p>(1) Valid without restriction</p> <p>Huntingdon Life Sciences Ltd. 2003. Assessment of biodegradability using the closed bottle method. Project ID CSS 006. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Biodegradation

Test Substance	2-Methyl, 2--Butene CAS No. 513-35-9 CAS Inventory Name: 2-Butene, 2-methyl-
Method/Guideline	OECD Guideline 301F
Year (guideline)	1992
Type (test type)	Ready Biodegradability: Manometric Respirometry Test
GLP (Y/N)	Yes
Year (study performed)	2003
Inoculum	Domestic activated sludge
Exposure Period	28 Days
Test Conditions	<p>Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 48/L and 46 mg/L, respectively. Blank test systems, which did not contain the test or positive control substance, were run concurrently in triplicate.</p> <p>The total suspended solids (TSS) of the activated sludge was determined to be 4.28 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The microbial count of the inoculum was 10⁵ CFU/mL. One liter of test medium, which was aerated for 24 hours with carbon dioxide free air, was added to each one liter respirometer flask. The test substance was weighed in an air tight syringe and injected into the test medium. The test system was sealed immediately after addition of the test substance. An aliquot of the positive control stock solution was added to the appropriate test flasks.</p> <p>An unacclimated activated sludge inoculum was used in this study. The inoculum was obtained from the Clinton Sanitary Wastewater Treatment Plant, Annandale, NJ, USA. The treatment plant receives domestic sewage.</p> <p>All test systems were placed on a Coordinated Environmental Services (CES) automated respirometer which automatically recorded the oxygen uptake in general agreement with the OECD guideline. The 28-day study was conducted at a temperature range of 22 ± 1°C.</p>
<ul style="list-style-type: none"> Note: Concentration preparation, vessel type, replication, test conditions. 	

Biodegradation

<p><u>Test Substance</u> Remarks</p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced from C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling point (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8.</p> <p>The three major components analysed were shown to be stable for the duration of testing in the study.</p> <p>The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (88%) was equivalent to 102% of the theoretical value (85.9%), which was based on the pre-shipment composition of the test substance.</p>
<p><u>Method</u> Method/guideline followed</p> <p>GLP Year Type Inoculum Concentration Contact time Test inoculum preparation</p>	<p>OECD Guideline 301D and EC Directive 92/69, C.4-E and EPA OPPTS 835.3110</p> <p>Yes 2002 Aerobic Domestic sewage effluent 2 mg/l related to test substance 28 days</p> <p>A sample of secondary effluent was collected on the day of the test from a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).</p>
<p>Study design</p>	<p>Eighteen bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/l, and the test substance at a nominal loading of 2 mg/l. The dissolved oxygen (DO) concentration in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. Four bottles were established for a concurrent five-day microbial inhibition assay, in which the biodegradation of the readily biodegradable reference substance, sodium benzoate, was examined in the presence of the test substance. DO concentration was measured in replicate bottles on Days 0 and 5.</p> <p>A further eighteen bottles were filled with mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/l, and the reference substance, sodium benzoate at a nominal loading of 5 mg/l. DO concentrations in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. All test systems were incubated at $22 \pm 2^\circ\text{C}$ in darkness.</p>

	<p>Theoretical oxygen demands for the test and reference substances were based on calculated empirical formulae and molecular weights. The study was initiated on 13 March 2002.</p>
<u>Results</u>	<p>A maximum of 9% biodegradation was measured (on Day 28) by the end of the Closed Bottle test.</p> <p>The mean Total Viable Count of the sample of final sewage effluent in the main test was 1.9×10^5 ColonyForming Units (CFU) per ml and the mean count in inoculated MSM on Day 0 was 1.4×10^4 CFU/ml.</p> <p>The presence of the test substance was not considered to have caused any inhibitory affect on the normal degradative activity of the microbial inoculum on the reference substance.</p>
Degradation	8 (\pm) % after 28 day(s)
Kinetic of test substance	<p>5 day(s) 3 and 3%</p> <p>7 day(s) 4 and 6%</p> <p>11 day(s) 0 and 2%</p> <p>14 day(s) 0 and 0%</p> <p>18 day(s) 8 and 8%</p> <p>21 day(s) 1 and 3%</p> <p>25 day(s) 4 and 6%</p> <p>28 day(s) 5 and 9%</p>
Control substance	Benzoic acid, sodium salt
Kinetic	<p>5 day(s) 69 and 70%</p> <p>28 day(s) 81 and 83%</p>
<u>Conclusions</u>	Not biodegradable
<u>Data Quality</u>	(1) Valid without restriction
<u>Reference</u>	Huntingdon Life Sciences Ltd. 2002. Assessment of biodegradability using the closed bottle method. Project ID CSS 016. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

Biodegradation

Test Substance	Pyrolysis C5s												
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68603-00-9	Distillates, petroleum, thermal cracked naphtha and gas oil												
68956-55-8	Hydrocarbons, C5-unsatd.												
Method/Guideline	OECD Guideline 301F												
Year (guideline)	1992												
Type (test type)	Ready Biodegradability: Manometric Respirometry Test												
GLP (Y/N)	Yes												
Year (study performed)	2003												
Inoculum	Domestic activated sludge												
Exposure Period	28 Days												
Test Conditions	<p>Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 49/L and 46 mg/L, respectively. Blank test systems, which did not contain the test or positive control substance, were run concurrently in triplicate.</p> <p>The total suspended solids (TSS) of the activated sludge was determined to be 4.28 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The microbial count of the inoculum was 10⁵ CFU/mL. One liter of test medium, which was aerated for 24 hours with carbon dioxide free air, was added to each one liter respirometer flask. The test substance was weighed in an air tight syringe and injected into the test medium. The test system was sealed immediately after addition of the test substance. An aliquot of the positive control stock solution was added to the appropriate test flasks.</p>												
Note: Concentration preparation, vessel type, replication, test conditions.													

<p>Test Conditions (cont.)</p> <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, replication, test conditions. <p>Results</p> <p>Units/Value:</p> <p>Note: Deviations from protocol or guideline analytical method.</p> <p>Conclusion</p> <p>Reliability</p> <p>Reference</p>	<p>An unacclimated activated sludge inoculum was used in this study. The inoculum was obtained from the Clinton Sanitary Wastewater Treatment Plant, Annandale, NJ, USA. The treatment plant receives domestic sewage.</p> <p>All test systems were placed on a Coordinated Environmental Services (CES) automated respirometer which automatically recorded the oxygen uptake in general agreement with the OECD guideline. The 28-day study was conducted at a temperature range of $22 \pm 1^\circ\text{C}$.</p> <p>Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test substance as calculated using results of an elemental analysis of the test substance.</p> <p>By day 14, >60% biodegradation of positive control was observed, which meets the guideline requirement. No deviations from the protocol occurred that affected the integrity of the study data.</p> <p>No measurable biodegradation was observed in triplicate test systems therefore the test substance cannot be considered readily biodegradable.</p> <table border="1" data-bbox="690 934 1299 1081"> <thead> <tr> <th></th> <th>% Degradation*</th> <th>Mean %</th> </tr> </thead> <tbody> <tr> <td>Degradation</td> <td></td> <td></td> </tr> <tr> <td><u>Sample</u></td> <td><u>(day 28)</u></td> <td><u>(day 28)</u></td> </tr> <tr> <td>Test Substance</td> <td>0, 0, 0</td> <td>0</td> </tr> <tr> <td>Na Benzoate</td> <td>91, 100, 89</td> <td>94</td> </tr> </tbody> </table> <p>* replicate data</p> <p>Not readily biodegradable</p> <p>(1) Reliable without restriction</p> <p>ExxonMobil Biomedical Sciences, Inc. 2004. Ready Biodegradability: Manometric Respirometry test. Study # 183994A</p>		% Degradation*	Mean %	Degradation			<u>Sample</u>	<u>(day 28)</u>	<u>(day 28)</u>	Test Substance	0, 0, 0	0	Na Benzoate	91, 100, 89	94
	% Degradation*	Mean %														
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<u>Sample</u>	<u>(day 28)</u>	<u>(day 28)</u>														
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Biodegradation

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed</p> <p>GLP Year Type Inoculum Concentration Contact time Test inoculum preparation</p> <p>Study design</p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Naphthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study. The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (86.7%) was equivalent to 103% of the theoretical value (84.1%), which was based on the pre-shipment composition of the test substance.</p> <p>OECD Guideline 301D and EC Directive 92/69, C.4-E and EPA OPPTS 835.3110</p> <p>Yes 2002 Aerobic Domestic sewage effluent 2 mg/l related to test substance 28 days A sample of secondary effluent was collected on the day of the test from a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).</p> <p>Eighteen bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/l, and the test substance at a nominal loading of 2 mg/l. The dissolved oxygen (DO) concentration in replicate bottles was measured on Days, 0, 5, 7, 11, 14, 18, 21, 25 and 28. Four bottles were established for a concurrent five-day microbial inhibition assay, in which the biodegradation of the readily biodegradable reference substance, sodium benzoate, was examined in the presence of the test substance. DO concentration was measured in replicate bottles on Days 0 and 5. A further eighteen bottles were filled with mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/l, and the reference substance, sodium benzoate at a nominal loading of 5 mg/l. DO concentrations in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. All test systems were incubated at $22 \pm 2^\circ\text{C}$ in darkness. Theoretical oxygen demands for the test and reference substances were based on calculated empirical formulae and molecular weights. The study was initiated on 15 October 2002.</p>
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<p><u>Results</u></p> <p>Degradation</p> <p>Kinetic of test substance</p> <p>Control substance</p> <p>Kinetic</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>A sample of secondary effluent was collected on the day of the test from a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).</p> <p>A maximum of 51% biodegradation was measured (on Day 28) by the end of the Closed Bottle test.</p> <p>The mean Total Viable Count of the sample of final sewage effluent in the main test was 1.6×10^5 Colony Forming Units (CFU) per ml and was diluted 1000-fold in the medium. The mean count of viable micorganisms in inoculated MSM on Day 0 was 2.7×10^3 CFU/ml.</p> <p>The presence of the test substance was not considered to have caused any inhibitory affect on the normal degradative activity of the microbial inoculum on the reference substance.</p> <p>51 (\pm) % after 28 day(s)</p> <p>5 day(s) 0 and 0% 7 day(s) 3 and 1% 11 day(s) 0 and 0% 14 day(s) 15 and 28% 18 day(s) 17 and 17% 21 day(s) 33 and 15% 25 day(s) 8 and 30% 28 day(s) 6 and 51%</p> <p>Benzoic acid, sodium salt</p> <p>5 day(s) 76 and 74% 28 day(s) 89 and 88%</p> <p>Not readily biodegradable</p> <p>(1) Valid without restriction</p> <p>Huntingdon Life Sciences Ltd. 2003. Assessment of biodegradability using the closed bottle method. Project ID CSS 026. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Biodegradation

Test Substance	Hydrotreated C5s								
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Method/Guideline:	OECD Guideline 301F								
Year (guideline)	1992								
Type (test type)	Ready Biodegradability: Manometric Respirometry Test								
GLP (Y/N)	Yes								
Year (study performed)	2003								
Inoculum	Domestic activated sludge								
Exposure Period	60 Days								
Test Conditions	<p>Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 48/L and 46 mg/L, respectively. Blank test systems, which did not contain the test or positive control substance, were run concurrently in triplicate.</p> <p>The total suspended solids (TSS) of the activated sludge was determined to be 4.28 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The microbial count of the inoculum was 10⁵ CFU/mL. One liter of test medium, which was aerated for 24 hours with carbon dioxide free air, was added to each one liter respirometer flask. The test substance was weighed in an air tight syringe and injected into the test medium. The test system was sealed immediately after addition of the test substance. An aliquot of the positive control stock solution was added to the appropriate test flasks.</p>								
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<p>Test Conditions (cont.)</p> <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, replication, test conditions. <p>Results</p> <p>Units/Value</p> <p>Note: Deviations from protocol or guideline analytical method.</p> <p>Conclusion</p> <p>Reliability</p> <p>Reference</p>	<p>An unacclimated activated sludge inoculum was used in this study. The inoculum was obtained from the Clinton Sanitary Wastewater Treatment Plant, Annandale, NJ, USA. The treatment plant receives domestic sewage.</p> <p>All test systems were placed on a Coordinated Environmental Services (CES) automated respirometer which automatically recorded the oxygen uptake in general agreement with the OECD guideline. The 60-day study was conducted at a temperature range of $22 \pm 1^\circ\text{C}$.</p> <p>Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test substance as calculated using results of an elemental analysis of the test substance.</p> <p>By day 14, >60% biodegradation of positive control was observed, which meets the guideline requirement. The positive control was terminated as 28 days. No deviations from the protocol occurred that affected the integrity of the study data.</p> <p>The test substance biodegradation run was extended beyond 28 days. The mean percent biodegradation of the test substance was determined to be 67% on day 60. The test substance did not achieve 60% biodegradation after 28 days therefore the test substance cannot be considered readily biodegradable.</p> <table border="0" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 40%;"></th> <th style="width: 30%; text-align: center;">% Degradation*</th> <th style="width: 30%; text-align: center;">Mean %</th> </tr> </thead> <tbody> <tr> <td colspan="3">Degradation</td> </tr> <tr> <td style="text-align: center;"><u>Sample</u></td> <td style="text-align: center;"><u>(day 28)</u></td> <td style="text-align: center;"><u>(day 28)</u></td> </tr> <tr> <td>Test Substance</td> <td style="text-align: center;">0, 19, 3</td> <td style="text-align: center;">11**</td> </tr> <tr> <td>Na Benzoate</td> <td style="text-align: center;">91, 100, 89</td> <td style="text-align: center;">93</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td style="text-align: center;"><u>Sample</u></td> <td style="text-align: center;"><u>(day 60)</u></td> <td style="text-align: center;"><u>(day 60)</u></td> </tr> <tr> <td style="text-align: center;">Test Substance</td> <td style="text-align: center;">67 **</td> <td style="text-align: center;">39,72,62</td> </tr> </tbody> </table> <p>* replicate data **Replicate # 1 omitted due to copper sulfate in the trap.</p> <p>Not readily biodegradable</p> <p>(1) Reliable without restriction</p> <p>ExxonMobil Biomedical Sciences, Inc. 2004. Ready Biodegradability: Manometric Respirometry test. Study # 183894A</p>		% Degradation*	Mean %	Degradation			<u>Sample</u>	<u>(day 28)</u>	<u>(day 28)</u>	Test Substance	0, 19, 3	11**	Na Benzoate	91, 100, 89	93	 			<u>Sample</u>	<u>(day 60)</u>	<u>(day 60)</u>	Test Substance	67 **	39,72,62
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HUMAN HEALTH ROBUST SUMMARIES**Acute Toxicity**

<p><u>Test Substance</u></p> <p><u>Method</u> Method/guideline followed Type (test type) GLP Year Species/Strain Sex No. of animals per sex per dose Vehicle Route of administration</p> <p>Test Conditions</p> <p><u>Results</u> LC₅₀ with confidence limits</p> <p>Remarks</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliability</p> <p><u>Reference</u></p>	<p>Isoprene, CAS# 78-79-5</p> <p>Other. Acute inhalation -LC₅₀ Pre-GLP 1969 Rat and mouse (strains not specified) Not specified Not specified Not applicable Inhalation (vapor)</p> <p>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.</p> <p>Rat LC₅₀ (4 hr) = 180 mg/L (64,620 ppm); confidence limits 130-181 mg/L (p≤0.05). Mouse LC₅₀ (2 hr) = 157 mg/L (56,363 ppm); confidence limits 129-252 mg/L (p≤0.05).</p> <p>No clinical observations or necropsy findings reported. Objective of study was to determine hydrocarbon concentrations in various tissues at lethal exposure concentrations.</p> <p>LC50 value reported to be 180 mg/L (64,620 ppm) in rats, 157 mg/L (56,363 ppm) in mice.</p> <p>Not assignable. Lethality study only; insufficient experimental detail to assess quality.</p> <p>Shugaev, B.B. (1969) Concentrations of Hydrocarbons in Tissues as a Measure of Toxicity. Arch. Environ. Health 18:878-882.</p>
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Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> <i>Test substance</i></p> <p><u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested Statistical Methods</p> <p>Remarks for Test Conditions</p> <p><u>Results</u> Genotoxic effects</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>Isoprene, CAS# 78-79-5 Purity >99%.</p> <p>OECD 471 Ames <i>Salmonella</i>/bacterial reverse mutation test (pre-incubation assay). Bacterial Yes 1986 <i>Salmonella</i> / TA98, TA100, TA1535, TA1537 With and without Rat and hamster liver S9 fraction 0.5 ml/plate Arochlor 1254-induced (500 mg/kg for 5 days) 0, 100, 333, 1000, 3333, 10000 ug/plate 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.</p> <p>The preincubation modification of the <i>Salmonella</i>/mammalian microsome assay was used to test isoprene in five different <i>Salmonella</i> 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.</p> <p>Negative Isoprene was not mutagenic in any of the five strains of <i>Salmonella</i> tested in the presence or absence of Arochlor-induced rat or hamster liver S9.</p> <p>Isoprene was not mutagenic in the Ames <i>Salmonella</i> mutagenicity test.</p> <p>(1) Reliable without restrictions. Evaluated as part of a NTP-sponsored interlaboratory study of 270 chemicals.</p> <p>Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986) <i>Salmonella</i> mutagenicity tests: II. Results from the testing of 270 chemicals. <i>Environ. Mutagen.</i> 8 (Suppl. 7): 1-119.</p>
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Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> Test substance</p> <p><u>Method</u> Method/guideline followed Type System of testing GLP Year Metabolic activation Concentrations tested Control groups and treatment</p> <p>Statistical Methods</p> <p>Remarks for Test Conditions</p> <p><u>Results</u> Genotoxic effects</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>Isoprene, CAS# 78-79-5 Purity >99%.</p> <p>OECD 479 <i>In vitro</i> Sister Chromatid Exchange (SCE) Assay in Mammalian Cells Chinese hamster ovary (CHO) cells Yes 1987 Aroclor 1254-induced Sprague-Dawley rat liver S9 50, 160, 500, 1600 ug/ml (without S9), or 160, 500, 1600, 5000 ug/ml (with S9) Solvent controls: dimethylsulfoxide; positive controls: Mitomycin-C (without S9), cyclophosphamide (with S9).</p> <p>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.</p> <p>Isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) both in the presence and absence of Aroclor 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 2nd-division metaphase cells were scored for frequency of SCEs/cell from each dose level.</p> <p>Negative No increases in SCEs were noted in cultured CHO cells treated with isoprene, with or without S9.</p> <p>Isoprene did not induce sister chromatid exchanges <i>in vitro</i> in cultures of Chinese hamster ovary cells.</p> <p>(1) Reliable without restrictions. Evaluated as part of a NTP-sponsored study of 108 chemicals.</p> <p>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. <i>Environ Mol. Mutagen</i> 10:1-175.</p>
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Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> Test substance</p> <p><u>Method</u> Method/guideline followed Type System of testing GLP Year Metabolic activation Concentrations tested Control groups and treatment</p> <p>Statistical Methods</p> <p>Remarks for Test Conditions</p> <p><u>Results</u> Genotoxic effects</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>Isoprene, CAS# 78-79-5 Purity >99%.</p> <p>OECD 473 <i>In vitro</i> Mammalian Chromosomal Aberration Test Chinese hamster ovary (CHO) cells Yes 1987 Aroclor 1254-induced Sprague-Dawley rat liver S9. 1600, 3000, 5000 ug/ml Solvent control: dimethylsulfoxide; positive controls: Mitomycin-C (without S9), cyclophosphamide (with S9). 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.</p> <p>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.</p> <p>Negative No increases in chromosomal aberrations were noted in cultured CHO cells treated with isoprene, with or without S9.</p> <p>Isoprene did not induce chromosomal aberrations <i>in vitro</i> in cultures of Chinese hamster ovary cells.</p> <p>(1) Reliable without restrictions. Evaluated as part of a NTP-sponsored study of 108 chemicals.</p> <p>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. <i>Environ Mol. Mutagen</i> 10:1-175.</p>
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Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period Statistical methods</p> <p>Remarks for Test Conditions</p> <p><u>Results</u> Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)</p> <p><u>Conclusions</u></p>	<p>Isoprene, CAS# 78-79-5 Purity >98%.</p> <p>OECD 475 Mammalian Bone Marrow Chromosomal Aberration Test Yes 1988 Mouse B6C3F1 15 male/group Inhalation (vapor) 0, 438, 1750, 7000 ppm 6 hours/day for 12 days 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</p> <p>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 for replication 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.</p> <p>Negative 7000 ppm >7000 ppm</p> <p>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</p>
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<p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>References</u></p>	<p>significant change in the percentage of bone marrow cells engaged in division, although the 7000 ppm group was slightly increased compared to the controls (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).</p> <p>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.</p> <p>(1) Reliable without restriction</p> <p>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. <i>Mutagenesis</i> 3(2):141-146.</p>
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Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> Remarks</p>	<p>2-Butene, 2-methyl CAS# 513-35-9 (2-methyl-2-butene, 85% purity)</p>
<p><u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain Metabolic activation Species and cell type Quantity Induced or not induced Concentrations tested Statistical Methods</p>	<p>OECD 471 Ames <i>Salmonella</i>/bacterial reverse mutation test (pre-incubation assay). Bacterial. Yes. 1980. <i>Salmonella typhimurium</i>/ TA98, TA100, TA1535, TA1537, TA1538 With and without. Rat liver S9 fraction. 0.5 ml/plate. Arochlor 1254-induced. 0, 0.2, 2, 20, 500, and 2000 ug/plate. 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, dose-related increase in any one strain/activation combination was interpreted as positive.</p>
<p>Remarks for Test Conditions</p>	<p>The preincubation modification of the <i>Salmonella</i>/mammalian microsome assay was tested in five different <i>Salmonella</i> 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.</p>
<p><u>Results</u> Genotoxic effects</p>	<p>Negative The test substance was not mutagenic in any of the five strains of <i>Salmonella</i> tested in the presence or absence of metabolic activation (rat liver S9).</p>
<p><u>Conclusions</u></p>	<p>The test substance was not mutagenic in the Ames <i>Salmonella</i> mutagenicity test.</p>
<p><u>Data Quality</u></p>	<p>(1) Reliable without restriction</p>
<p><u>Reference</u></p>	<p>Dean, B.J., Brooks, T.M., Hodson-Walker, G., and Hutson, D.H. (1985). Genetic toxicology testing of 41 industrial chemicals. <i>Mutation Research</i> 153:57-77.</p>

Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> Remarks</p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pryolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin / Olefin / Naphthene / Aromatic PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-entene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.</p>
<p><u>Method</u> Method / guideline followed GLP Year Type System</p>	<p>Directive 2000/32/EC, B.13/14; OECD Guideline 471; OPPTS 770.5100 Yes 2002 Bacterial reverse mutation assay Bacterial - <i>Salmonella typhimurium</i>, strains TA1535, TA1537, TA98 and TA100 and <i>E. coli</i>, strain WP2uvrA/pKM101 (CM891) were used.</p>
<p>Concentrations</p>	<p>0.,85, 0.27, 0.085, 0.027, 0.0085, 0.0027, 00085 % v/v (8500, 2700, 850, 270, 8.5 ppm) - test 1 0.85, 0.425, 0.213, 0.107, 0.054% v/v (8500, 4250, 2130, 1070, 540 ppm) test 2</p>
<p>Metabolic activation</p>	<p>With and without S9 mix (10% v/v in test 1, 20% v/v in test 2) prepared from Aroclor-induced rat liver.</p>
<p>Study design</p>	<p>Concentrations of the test substance up to 0.85% v/v (8500 ppm; 50% of the Lower Explosive Limit) were tested in the mutation tests in vapour phase. Agar plates, seeded with the tester strains, were exposed to the test substance for 48 hours at 37°C, the incubated in the absence of the test substance for a further 24 hours. Revertant colony numbers were counted after incubation. The following positive control chemicals were used: (a) Requiring metabolic activation (response relative to untreated controls); Benzo[a]pyrene, 5 µg/plate: TA98 6-13 x, TA100 4-6 x, TA1537 7-15 x; 2-Aminoanthracene, 2 µg/plate: TA1535 4-16x; 2-Aminoanthracene, 10 µg/plate: CM891 8-10 x. (b) Direct-acting (response relative to untreated controls): 2-Nitrofluorene, 1 µg/plate: TA98 9-13 x; Sodium azide, 0.5 µg/plate: TA100 4-6 x, TA1535 16-32 x; 9-Aminoacridine, 50 µg/plate: TA1537 26-37 x; AF-2, 0.05 µg/plate: CM891 3 x; Dichloromethane, 7.5% v/v in vapour phase: TA98 34 x; TA100 9-14 x. All responses were within historical data ranges.</p>
<p><u>Results</u></p>	<p>No signs of toxicity observed towards the tester strains in either</p>

<u>Conclusions</u>	mutation test. All bacterial lawns were normal.
<u>Data Quality</u>	Negative
<u>Reference</u>	(1) Valid without restriction
	Huntingdon Life Sciences Ltd. 2002. Bacterial Reverse Mutation Test. Project ID CSS 019. Huntingdon Life Sciences Ltd., Cambridgeshire, England

Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> Remarks</p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in this study</p>
<p><u>Method</u> Method / guideline followed</p> <p>GLP Year Type System</p>	<p>Directive 2000/32/EC, B, B.13/14; OECD Guideline 471; OPPTS 770.5100</p> <p>Yes 2002 Bacterial reverse mutation assay Bacterial - <i>Salmonella typhimurium</i>, strains TA1535, TA1537, TA98 and TA100 and <i>E. coli</i>, strain WP2uvrA/pKM101 (CM891) were used.</p>
<p>Concentrations</p>	<p>0.525, 0.166, 0.0525, 0.0166, 0.00525, 0.00166, 0.000525 %v/v (5250, 1660, 525, 166, 52.5, 16.6, 5.25 ppm) - test 1 0.525, 0.263, 0.131, 0.066, 0.033% v/v (5250, 2360, 1310, 660, 330 ppm) - test 2</p>
<p>Metabolic activation</p>	<p>With and without S9 mix (10% v/v in test 1, 20% v/v in test 2) prepared from Aroclor-induced rat liver.</p>
<p>Study design</p>	<p>Concentrations of the test substance up to 0.525% v/v (5250 ppm; 50% of the Lower Explosive Limit) were tested in the mutation tests in vapour phase. Agar plates, seeded with the tester strains, were exposed to the test substance for 48 hours at 37°C, then incubated in the absence of the test substance for a further 24 hours. Revertant colony numbers were counted after incubation.</p> <p>The following positive control chemicals were used:</p> <p>(a) Requiring metabolic activation (response relative to untreated control): Benzo[a]pyrene, 5 µg/plate: TA98 8-9 x, TA100 3-4 x, TA1537 9-10 x; 2-Aminoanthracene, 2 µg/plate: TA1535 4-13 x; 2-Aminoanthracene, 10 µg/plate: CM891 7-8 x.</p> <p>(b) Direct-acting (response relative to untreated controls): 2-Nitrofluorene, 1 µg/plate: TA98 9-23 x; Sodium azide, 0.5 µg/plate: TA100 4-5 x, TA1535 22-27 x; 9-Aminoacridine, 30 µg/plate: TA1537 14-21 x; AF-2, 0.05 µg/plate: CM891 8-9x; Dichloromethane, 7.5% v/v in vapour phase: TA98 36-51 x; TA100 18 x.</p> <p>All responses were within historical data ranges, except dichloromethane, where responses exceeded the historical data range.</p>
<p><u>Results</u></p>	<p>No signs of toxicity observed towards the tester strains in either</p>

<p><u>Conclusions</u></p> <p><u>Data quality</u></p> <p><u>Reference</u></p>	<p>mutation test. All bacterial lawns were normal. No increases in reversion to prototrophy were obtained in any of the tester strains following exposure to the test substance.</p> <p>Negative</p> <p>(1) Valid without restriction</p> <p>Huntingdon Life Sciences Ltd. 2002. Bacterial Reverse Mutation Test. Project ID CSS 009. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p>	<p>Isoprene, CAS# 78-79-5 Purity >98%.</p>
<p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period Statistical methods</p>	<p>Other. <i>In vivo</i> Sister Chromatid Exchange (mouse bone marrow cytogenetics study) Yes 1988 Mouse B6C3F1 15 male/group Inhalation (vapor) 0, 438, 1750, 7000 ppm 6 hours/day for 12 days The frequencies of sister chromatid exchanges (SCEs) were analyzed for increasing trend by the one-tailed Cochran-Armitage 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</p>
<p>Remarks for Test Conditions</p>	<p>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.</p>
<p><u>Results</u> Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)</p>	<p>Positive <438 ppm 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.. There were no significant clinical signs or mortality throughout the study.</p>
<p><u>Conclusions</u></p>	<p>Isoprene was found to be genotoxic and cytotoxic to mouse bone marrow <i>in vivo</i> - inducing SCE, inhibiting cellular</p>

<p><u>Data Quality</u></p> <p><i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>proliferation, and suppressing the rate of erythropoiesis. The lack of significant difference in SCEs among the three exposed groups suggests a saturation of the metabolic capacity of male mice to form reactive species.</p> <p>(1) Reliable without restrictions. NTP-sponsored study.</p> <p>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. <i>Mutagenesis</i> 3(2):141-146.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p>	<p>Isoprene, CAS# 78-79-5 Purity >98%</p>
<p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period Statistical methods</p>	<p>OECD 475 Mammalian Bone Marrow Chromosomal Aberration Test Yes 1988 Mouse B6C3F1 15 male/group Inhalation (vapor) 0, 438, 1750, 7000 ppm 6 hours/day for 12 days The frequencies of chromosomal aberrations (Abs) were analyzed for increasing trend by the one-tailed Cochran-Armitage 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</p>
<p>Remarks for Test Conditions</p>	<p>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 for replication 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.</p>
<p><u>Results</u> Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)</p>	<p>Negative 7000 ppm >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</p>

<p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>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 controls (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).</p> <p>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.</p> <p>(1) Reliable without restrictions. NTP-sponsored study.</p> <p>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. <i>Mutagenesis</i> 3(2):141-146.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p>	<p>Isoprene, CAS# 78-79-5 Purity >98%</p>
<p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period Statistical methods</p>	<p>OECD 474 Mammalian Erythrocyte Micronucleus Test Yes 1988 Mouse B6C3F1 15 male/group Inhalation (vapor) 0, 438, 1750, 7000 ppm 6 hours/day for 12 days 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.</p>
<p>Remarks for Test Conditions</p>	<p>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.</p>
<p><u>Results</u> Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)</p>	<p>Positive <438 ppm. 438 ppm.</p> <p>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 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.</p>
<p><u>Conclusions</u></p>	<p>Isoprene was found to be genotoxic to mouse bone marrow <i>in vivo</i> by inducing increased MN in the peripheral blood of male mice. Suppression of erythropoiesis was suggested by decreased percentage of PCEs.</p>

<p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>(1) Reliable without restrictions. NTP-sponsored study.</p> <p>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. <i>Mutagenesis</i> 3(2):141-146.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period Statistical methods</p> <p>Remarks for Test Conditions</p> <p><u>Results</u> Genotoxic effects</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p>	<p>Isoprene, CAS# 78-79-5 Purity >99.7%.</p> <p>Other Rat Lung Fibroblast Micronucleus Test Yes 1997 Rat Fischer 344 10 male and 10 female/group Inhalation (vapor) 0, 220, 700, or 7000 ppm 6 hours/day, 5 days/week, for 4 weeks 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.</p> <p>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.</p> <p>Negative</p> <p>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.</p> <p>No significant increase in the frequency of micronucleated lung fibroblasts was observed in male and female rats exposed to isoprene for 4 weeks.</p> <p>(1) Reliable with restrictions. Non-standard method, but comparable to guideline study. Conducted as part of NTP two-year carcinogenicity study.</p>
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<u>Reference</u>	National Toxicology Program (1997). Toxicology and Carcinogenesis Studies of Isoprene (CAS No. 78-79-5) in F344/N Rats (Inhalation Studies). Report No. TR-486.
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method / guideline followed</p> <p>GLP Year Type Species Sex Strain Route of administration Exposure period Doses</p> <p>Study design</p> <p><u>Results</u></p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pryolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin / Olefin / Naphthene / Aromatic PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-entene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.</p> <p>OECD Guideline 474 (1997), US EPA OPPTS 870.5395 (1998) and EC Directive 2000/32/EC, L 136/50</p> <p>Yes. 2002. Micronucleus assay Mouse Male CD-1 Inhalation 2 x 6 hours approximately 24 hours apart 2000, 4000, and 8000 parts per million (ppm)</p> <p>Groups of seven male CE-1 mice (approximately 32g bodyweight) were exposed to Hydrotreated C5s for two 6-hour whole body exposure periods, on consecutive days, at target concentrations of 2000, 4000 and 8000 (ppm). Compressed air was used to generate atmospheres for both negative control and test chemical exposed groups. Gas chromatography was used to measure the concentrations of Hydrotreated C5s in the test atmospheres. Negative control animals were exposed using compressed air. A positive control group (5 animals) were doses once only by oral gavage with mitomycin C at 12 mg/kg. All animals were sacrificed approximately 24 hours after the second exposure period (24 hours after the oral dose for the positive control group) and bone marrow smears prepared. Smears were examined to evaluate the incidence of micronuclei (MN) in 2000 immature erythrocytes (MIE) per animal. The proportion of MIE was assessed by examination of at least 1000 erythrocytes.</p> <p>No statistically significant increase in the incidence of micronucleated MIE were observed in the Hydrotreated C5s exposed animals compared with the negative control values (Linear-by-Linear association test and exact one-tailed pairwise permutation test). The positive control treatment induced a significant increase. A small statistically significant decrease in the proportion of immature erythrocytes was observed in animals exposed to Hydrotreated C5s. The Jonckheere's test for trend was</p>
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	<p>significant with Groups 1 to 4 included ($P < 0.01$). When data from the high exposure group (8000 ppm) was excluded the trend was not significant ($P > 0.01$). There were no statistically significant results of the one-tailed Wilcoxon pairwise test for a decrease in the proportion of MIE, from negative control values. The proportion of immature erythrocytes was within the normal range of variability for this species and the decrease was not considered to be of any biological importance. Statistical significance was declared at the 1% level for all tests.</p>
<u>Conclusion</u>	Negative
<u>Data quality</u>	(1) valid without restriction
<u>Reference</u>	Huntingdon Life Sciences Ltd. 2002. Mouse Micronucleus Test. Project ID CSS 020. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method / guideline followed</p> <p>GLP Year Type Sex Route of administration Exposure period</p> <p>Study design</p> <p><u>Results</u></p> <p><u>Conclusion</u></p> <p><u>Data quality</u></p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in this study.</p> <p>OECD Guideline 474 (1997), US EPA OPPTS 870.5395 (1998) and EC Directive 2000/32/EC, L 13650</p> <p>Yes 2002 Mouse Male Inhalation 2 x 6 hours approximately 24 hours apart 40, 125, and 500 parts per million (ppm)</p> <p>Groups of seven male CD-1 mice (approximately 32g bodyweight) were exposed to Pyrolysis C5s for two 6-hour whole body exposure period, on consecutive days, at target concentrations of 40, 150 and 500 (ppm). Compressed air was used to generate atmospheres for both negative control and test chemical exposed groups. Gas chromatography was used to measure the concentrations of Pyrolysis C5s in the test atmospheres. Negative control animals were exposed using compressed air. A positive control group (5 animals) were dosed once only by oral gavage with mitomycin C at 12 mg/kg. All animals were sacrificed approximately 24 hours after the second exposure period (24 hours after the oral dose for the positive control group) and bone marrow smears prepared. Smears were examined to evaluate the incidence of micronuclei (MN) in 2000 polychromatic erythrocytes (PCE) per animals. The proportion of PCE was assessed by examination of at least 1000 erythrocytes.</p> <p>No statistically significant increase in the incidence of micronucleated PCE were observed in the Pyrolysis C5s exposed animals compared with the negative control values (Linear-by-Linear association test and exact one-tailed pairwise permutation test). The positive control treatment induced a significant increase. Statistical significance was declared at the 1% level for all tests.</p> <p>Negative</p> <p>(1) valid without restriction</p>
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<u>Reference</u>	Huntingdon Life Sciences Ltd 20002. Mouse Micronucleus Test. Project ID CSS 010. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p>	<p>Isoamylene, CAS# 26760-64-5 (90% 2-Butene, 2-methyl; 10% 1-Butene, 2-methyl)</p>
<p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period No. of animals per dose Control groups and treatment Statistical methods</p>	<p>OECD 474 Mammalian erythrocyte micronucleus test Yes 1990 Mouse B₆C₃F₁ Males Inhalation (vapor) 0, 1034, 3258 or 10,350 ppm (analytical mean concentrations). 6 hours/day for 2 consecutive days 10 males/exposure level 10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control) 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.</p>
<p>Remarks for Test Conditions</p>	<p>Ten male B₆C₃F₁ 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.</p>
<p><u>Results</u></p>	<p>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%.</p>

<p><u>Conclusions</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>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 B₆C₃F₁ mice.</p> <p>(1) Reliable without restriction</p> <p>ExxonMobil Biomedical Sciences, Inc. (1990). In Vivo Mammalian Bone Marrow Micronucleus Assay. Unpublished study.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p>	<p>2-Butene, 2-methyl CAS# 513-35-9 (2-methyl-2-butene, >99.2% purity)</p>
<p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period No. of animals per dose Control groups and treatment Statistical methods</p>	<p>OECD 474 Mammalian erythrocyte micronucleus test. Yes 1991 Mouse B₆C₃F₁ Males Inhalation (vapor) 0, 1005, 3207, or 9956 ppm (analytical mean concentrations) 6 hours/day for 2 consecutive days 10 males/exposure level. 10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control) 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.</p>
<p>Remarks for Test Conditions</p>	<p>Ten male B₆C₃F₁ 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.</p>
<p><u>Results</u></p>	<p>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%.</p>
<p><u>Conclusions</u></p>	<p>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 B₆C₃F₁ mice.</p>

<p><u><i>Data Quality</i></u></p> <p><u><i>References</i></u></p>	<p>(1) Reliable without restriction</p> <p>ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period No. of animals per dose Control groups and treatment Statistical methods</p> <p>Remarks for Test Conditions</p> <p><u>Results</u></p> <p><u>Conclusions</u></p>	<p>2-Butene, 2-methyl CAS# 513-35-9 (2-methyl-2-butene, >99.2% purity)</p> <p>OECD 474 Mammalian erythrocyte micronucleus test Yes 1991 Rat CrICDBR Males Inhalation (vapor) 0, 1005, 3207, or 9956 ppm (analytical mean concentrations) 6 hours/day for 2 consecutive days 10 males/exposure level 10 males exposed to air (negative control) 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.</p> <p>Ten male CrICDBR 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.</p> <p>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 from 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.</p> <p>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.</p>
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<p><u>Data Quality</u></p> <p><u>References</u></p>	<p>(2) Reliable with restrictions. No concurrent positive control was used.</p> <p>ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p>	<p>1-Butene, 2-methyl CAS# 26760-64-5 (2-methyl-1-butene, >99.2% purity)</p>
<p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period No. of animals per dose Control groups and treatment Statistical methods</p>	<p>OECD 474 Mammalian erythrocyte micronucleus test Yes 1991 Mouse B₆C₃F₁ Males Inhalation (vapor) 0, 1038, 3312, or 10,116 ppm (analytical mean concentrations) 6 hours/day for 2 consecutive days 10 males/ exposure level 10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control) 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.</p>
<p>Remarks for Test Conditions</p>	<p>Ten male B₆C₃F₁ mice (weighing 24-30 g, approximately 7-8 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.</p>
<p><u>Results</u></p>	<p>A dose-related increase in mean micronucleated PCEs was observed (2.4, 3.7, 3.6, and 4.6 at 0, 1038, 3312 and 10,116 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 10,116 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%.</p>
<p><u>Conclusions</u></p>	<p>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 B₆C₃F₁ mice.</p>

<p><u><i>Data Quality</i></u></p> <p><u><i>References</i></u></p>	<p>(1) Reliable without restriction</p> <p>ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period No. of animals per dose Control groups and treatment Statistical methods</p> <p>Remarks for Test Conditions</p> <p><u>Results</u></p> <p><u>Conclusions</u></p> <p><u>Data Quality</u></p> <p><u>References</u></p>	<p>1-Butene, 2-methyl CAS# 26760-64-5 (2-methyl-1-butene, >99.2% purity)</p> <p>OECD 474 Mammalian erythrocyte micronucleus test Yes 1991 Rat CrICDBR Males Inhalation (vapor) 0, 1038, 3312, or 10,116 ppm (analytical mean concentrations) 6 hours/day for 2 consecutive days 10 males/exposure level 10 males exposed to air (negative control) 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.</p> <p>Ten male CrICDBR rats (weighing 337-414 g, approximately 10-11 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.</p> <p>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.</p> <p>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 CrICDBR rats.</p> <p>(2) Reliable with restrictions. No concurrent positive control was used.</p> <p>ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay. Unpublished study.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period No. of animals per dose Control groups and treatment Statistical methods</p> <p>Remarks for Test Conditions</p> <p><u>Results</u></p> <p><u>Conclusions</u></p> <p><u>Data Quality</u></p> <p><u>References</u></p>	<p>Isoamylene CAS# 26760-64-5 (~92% 2-Butene, 2-methyl; ~7% 1-Butene, 2-methyl)</p> <p>OECD 474.Mammalian erythrocyte micronucleus test Yes 1991 Mouse B₆C₃F₁ Males Inhalation (vapor) 0, 1034, 3266 or 10,097 ppm (analytical mean concentrations) 6 hours/day for 2 consecutive days 10 males/exposure level 10 males exposed to air (negative control), 10 males exposed to 1000 ppm 1,3 butadiene (positive control) 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.</p> <p>Ten male B₆C₃F₁ 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.</p> <p>The test substance induced statistically significant (p<0.01) and dose-related 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, 51.4, and 34.6% at 0, 1034, 3266 and 10,097 ppm. The %PCEs for the positive control was 43.7%.</p> <p>Under the conditions of this study, inhalation exposure to 3266 and 10,097 ppm of the test substance induced statistically significant increases in micronucleated polychromatic erythrocytes in male B₆C₃F₁ mice.</p>
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<p><u><i>Data Quality</i></u></p> <p><u><i>References</i></u></p>	<p>(1) Reliable without restriction</p> <p>ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay. Unpublished study.</p>
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period No. of animals per dose Control groups and treatment Statistical methods</p> <p>Remarks for Test Conditions</p> <p><u>Results</u></p> <p><u>Conclusions</u></p>	<p>Isoamylene CAS# 26760-64-5 (~92% 2-Butene, 2-methyl; ~7% 1-Butene, 2-methyl).</p> <p>OECD 474 Mammalian erythrocyte micronucleus test Yes 1991 Rat CrICDBR Males Inhalation (vapor) 0, 1034, 3266 or 10,097 ppm (analytical mean concentrations) 6 hours/day for 2 consecutive days 10 males/exposure level 10 males exposed to air (negative control) 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.</p> <p>Ten male CrICDBR rats (weighing 348-447 g, approximately 11-12 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.</p> <p>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 (0-4) 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. .</p> <p>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.</p>
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<p><u><i>Data Quality</i></u></p> <p><u><i>References</i></u></p>	<p>(2) Reliable with restrictions. No concurrent positive control was used.</p> <p>ExxonMobil Biomedical Sciences, Inc. (1991). In Vivo Mammalian Bone Marrow Micronucleus Assay - Inhalation Dosing Method. Unpublished study.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Isoprene, CAS# 78-79-5
Remarks	Purity >99%.
<u>Method</u>	Other
Method/guideline followed	2-week inhalation study
Test type	Yes
GLP	1990
Year	Rat and mouse
Species	F344 rats and B6C3F1 mice
Strain	Inhalation (vapor)
Route of administration	2 weeks
Duration of test	0, 438, 875, 1750, 3500, or 7000 ppm
Doses/concentration levels	20 male, 20 female per group
Sex	6 hours/day
Exposure period	5 days/week
Frequency of treatment	20 male, 20 female, air-only exposed
Control group and treatment	Not applicable
Post exposure observation period	Group mean body weights, organ weights, organ weight ratios, and clinical pathology results compared to controls by Dunnett's t-test.
Statistical methods	
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.
<u>Results</u>	
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

<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>atrophy of the testis and thymus, cytoplasmic vacuolization of the liver, olfactory epithelial degeneration in the nasal cavity, and epithelial hyperplasia in the forestomach.</p> <p>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.</p> <p>(1) Reliable without restrictions. Comparable to guideline study (OECD 412).</p> <p>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.</p>
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Repeated Dose Toxicity

<p><u>Test Substance</u> Remarks</p>	<p>Isoprene, CAS# 78-79-5 Purity >99%.</p>
<p><u>Method</u> Method/guideline followed Test type GLP Year Species Strain Route of administration Duration of test Doses/concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p>	<p>Other 13-week inhalation study Yes 1994 Rat and mouse F344 rats and B6C3F1 mice Inhalation (vapor) 13 weeks 0, 70, 220, 700, 2200, or 7000 ppm 10 male, 10 female per group 6 hours/day 5 days/week 10 male, 10 female, air-only exposed Not applicable Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.</p>
<p>Test Conditions</p>	<p>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.</p>
<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL)</p>	<p>7000 ppm rats, 220 ppm mice. >7000 ppm rats, 700 ppm mice.</p>
<p>Remarks</p>	<p>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 10/10 male mice at 7000 ppm. The male mice exposed to 7000 ppm exhibited testicular weights reduced 35% compared to the</p>

<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>controls.</p> <p>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.</p> <p>(1) Reliable without restrictions. Comparable to guideline study (OECD 413).</p> <p>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. <i>Cancer Res.</i> 54:5333-5339.</p>
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Repeated Dose Toxicity

<p><u>Test Substance</u> Remarks</p>	<p>Isoprene, CAS# 78-79-5 Purity >99%.</p>
<p><u>Method</u> Method/guideline followed Test type GLP Year Species Strain Route of administration Duration of test Doses/concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p>	<p>Other 26-week inhalation study Yes 1994 Rat and mouse F344 rats and B6C3F1 mice Inhalation (vapor) 26 weeks 0, 70, 220, 700, 2200, or 7000 ppm 40 male rats and 40 male mice per group 6 hours/day 5 days/week 40 male rats and 40 male mice, air-only exposed 26-week post-exposure recovery period Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.</p>
<p>Test Conditions</p>	<p>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.</p>
<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL)</p>	<p>>7000 ppm rats, 70 ppm mice. 7000 ppm rats, 700 ppm mice.</p>
<p>Remarks</p>	<p>The only effect observed in the male rats after 26 weeks of exposure was interstitial cell hyperplasia of the testis (10/10) 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). Survival of mice was reduced in the 7000 ppm group; early deaths were attributed to various neoplastic lesions and moribund sacrifices due to hindlimb paralysis. In male mice, incidences of malignant neoplastic lesions in the liver, lung, forestomach, and harderian</p>

<p><u>Conclusions</u></p> <p><u>Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>gland were significantly increased following the 26-week exposure and 26-week recovery periods at 700 ppm and higher exposures. Non-neoplastic lesions were observed in male mice exposed to isoprene and included spinal cord degeneration (≥ 70 ppm) and degeneration of the olfactory epithelium (≥ 220 ppm). Slight increases in testicular atrophy, epithelial hyperplasia of the forestomach, partial hindlimb paralysis and a nonresponsive macrocytic anemia were also seen in male mice.</p> <p>Selected non-neoplastic lesions were as follows (0, 70, 220, 700, 2200, 7000 ppm) -</p> <p><u>After 26 weeks exposure:</u> Nasal turbinates/olfactory epithelial degeneration - 0/10, 0/10, 0/10, 1/10, 1/10, 10/10. Testes/atrophy - 0/10, 0/10, 0/10, 0/10, 1/10, 5/10. Spinal cord/degeneration - 0/10, 0/10, 0/10, 0/10, 1/10, 10/10.</p> <p><u>After 26 weeks recovery:</u> Nasal turbinates/olfactory epithelial degeneration - 1/30, 2/30, 5/29, 11/30, 25/30, 28/28. Testes/atrophy - 0/30, 0/30, 0/29, 0/30, 0/30, 3/29. Spinal cord/degeneration - 4/30, 20/30, 19/29, 17/29, 13/28.</p> <p>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).</p> <p>(1) 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.</p> <p>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.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Isoprene, CAS# 78-79-5
Remarks	Purity >99.7%.
<u>Method</u>	Other
Method/guideline followed	2-year carcinogenicity study
Test type	Yes
GLP	1997
Year	Rat
Species	Fisher 344
Strain	Inhalation (vapor)
Route of administration	104 weeks
Duration of test	0, 220, 700, or 7000 ppm
Doses/concentration levels	50 male, 50 female per group
Doses/concentration levels	6 hours/day
Sex	5 days/week for 104 weeks
Exposure period	50 male, 50 female, exposed to air only
Frequency of treatment	None
Control group and treatment	Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Urine data was analyzed by nonparametric methods.
Post exposure observation period	
Statistical 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.
<u>Results</u>	
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 (12/50, 19/50, 17/50) were significantly greater than those in the chamber control groups (1/50 males, 7/50 females). The incidences of renal tubule adenoma in 7,000 ppm males (6/50) and of renal tubule hyperplasia in 700 ppm and 7,000 ppm males (6/50, 8/50) were significantly greater than those in the chamber controls (0/50). The severity of kidney nephropathy was slightly increased in 7,000 ppm males

<p><u>Conclusions</u> (Olefins Panel)</p> <p><u>Conclusions</u> (Study Authors)</p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>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 (11/50).</p> <p>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.</p> <p>There was clear evidence of carcinogenic activity in male rats based on increased incidences of mammary gland fibroadenoma 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.</p> <p>(1) Reliable without restrictions.</p> <p>National Toxicology Program (1997). Toxicology and Carcinogenesis Studies of Isoprene (CAS No. 78-79-5) in F344/N Rats (Inhalation Studies). Report No. TR-486.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Isoprene, CAS# 78-79-5
Remarks	Purity >99.0%.
<u>Method</u>	Other
Method/guideline followed	2-year carcinogenicity study
Test type	Yes
GLP	1996
Year	Mouse
Species	B6C3F ₁
Strain	Inhalation (vapor)
Route of administration	105 weeks
Duration of test	0, 10, 70, 140, 280, 700, 2200 ppm
Doses/concentration levels	50 male, 50 female per group
Sex	4 or 8 hours/day
Exposure period	Variable - 5 days/week for 20, 40, or 80 weeks
Frequency of treatment	50 male, 50 female, exposed to air only
Control group and treatment	Variable - animals held following exposures until week 96 or 105
Post exposure observation period	Body weights, organ weights and hematology data were evaluated by analysis of variance (ANOVA) followed by Duncan's new multiple range test. Incidences of tumor types were analyzed using Fischer's exact test applied to each combination of exposure group and tumor type.
Statistical methods	
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.
<u>Results</u>	
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

Repeated Dose Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method / guideline followed Test type</p> <p>GLP Year Species Strain Route of administration Duration of test Doses / concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p>	<p>2-methyl-2-butene (CAS number: 513-35-9). The test substance was stable for the duration of all studies performed at the test house</p> <p>OECD 422 4 week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats (Toxicity phase) Yes 2001 / 2002 Rat Cri:CD[®] (Sprague-Dawley) IGS BR Inhalation (gas) 28 days 0, 580, 2000, or 7000 ppm 12 males, 12 females per dose group for main study group 6 hours / day 7 days / week 12 males, 12 females, air-only exposed Not applicable All statistical analyses were carried out separately for males and females.</p> <p>Data relating to food consumption was analysed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. The following data types were analysed at each timepoint separately:- Rearing and activity counts. Bodyweight (FOB) and body temperature. Grip strength, landing footsplay and motor activity. Bodyweight, using gains over appropriate study periods. Food consumption, over appropriate study periods, using cumulative cage totals. Blood chemistry and haematology. Organ weights, absolute and / or adjusted for terminal bodyweight. Pathological findings, for the number of animals with and without each finding. For categorical data, including rearing and activity counts and pathological findings, the proportion of animals was analysed using Fisher's Exact test (Fisher 1973) for each treated group versus the control. For continuous data, Bartlett's test (Bartlett 1937) was first applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the Control group, incorporating adjustment for multiple comparisons where necessary. The following sequence of statistical tests was used for bodyweight (FOB), body temperature, grip strength, landing for splay and motor activity, bodyweight, food consumption, organ weight, and clinical pathology data. If 75% of the data (across all groups) were the same value, for</p>
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<p>Test Conditions</p>	<p>example c, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions (Mantel 1963) and also pairwise Fisher's Exact tests (Fisher 1973) for each dose group against the control both for 1) values $<c$ versus values $>=c$, and for 2) values $<=c$ versus values $>c$, as applicable. If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Williams' test for a monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead.</p> <p>If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Shirley's test for a monotonic trend (Shirley 1977) was applied. If the H1 test was significant, suggest that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead.</p> <p>For organ weight data, analysis of variance was initially performed using terminal bodyweight as covariate. If the within group relationship between organ weight and bodyweight was significant at the 10% level (Angervall and Carlstrom, 1963), then the treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.</p> <p>Significant differences between Control and treated groups were expressed at the 5% ($p<0.05$) or 1% ($p<0.01$) level. Williams test is denoted by '*'; t tests are denoted by '+', Dunnett's test is denoted by '*-' and Shirley's test by 'initially performed using terminal bodyweight as covariate. If the within group relationship between organ weight and bodyweight was significant at the 10% level (Angervall and Carlstrom, 1963), then the treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.</p> <p>Significant differences between Control and treated groups were expressed at the 5% ($p<0.05$) or 1% ($p<0.01$) level. Williams test is denoted by '*'; t tests are denoted by '+', Dunnett's test is denoted by '*-' and Shirley's test by '±'.</p> <p>Groups of 12 male and 12 female CD rats were exposed to the test material as a gas daily by inhalation for six hours / day at exposure levels f 0, 580, 2000, or 7000 ppm. In this main study (repeated-exposure general toxicity) males and females were exposed for 28 days. During the study, clinical condition, detailed functional observational battery, motor activity, bodyweight, food consumption, haematology, blood chemistry, organ weight and macroscopic and microscopic pathology investigations were undertaken. The study also contained reproductive / developmental toxicity satellite groups (summarized separately).</p>
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<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>580 ppm Not applicable The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations.</p> <p>Toxicity Phase</p> <p>Clinical signs during exposure included half-closed eyes on Day 1 at 2000 and 7000 ppm, and a lower level of response to external stimuli. This later finding also occurred on one further occasion at 7000 ppm. There were no signs considered reflective of a general systemic effect observed during routine clinical examination or during the functional observational battery. There was a slightly lower bodyweight gain at 7000 ppm, and slightly longer clotting times at 2000 ppm (prothrombin time for females) and 7000 ppm (prothrombin times for both sexes and activated partial thromboplastin time for males). Cholesterol levels were increased amongst females exposed to 7000 ppm but in the absence of any further effects in the clinical chemistry parameters or the males this is of uncertain significance.</p> <p>Pathological changes were noted amongst high dose females in the liver, evidenced as an increased organ weight and minimal centrilobular hepatocyte hypertrophy. There was a decreased incidence of extramedullary haemopoiesis of the spleen of high dose animals, an increase in goblet cell hyperplasia in the nasal passages of high dose males, and, amongst high and intermediate dose males, a slight increase in severity of myocardial inflammatory heart lesions and cortical / medullary tubular basophila in the kidneys.</p> <p>Slight effects on general systemic toxicity due to the test substance were apparent amongst animals receiving 7000 ppm, and to a lesser extent at 2000 ppm. The no effect level of the test substance for the general systemic toxicity to rats for 28 days inhalation administration was 580 ppm.</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd., 2004. 4-week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats Project ID CSS 002. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Repeated Dose Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method / guideline followed Test type</p> <p>GLP Year Species Strain Route of administration Duration of test Doses / concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin / Olefin / Naphthene / Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), dis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance 20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9.</p> <p>The test substance was considered to be stable for the duration of the study.</p> <p>OECD 422 4 week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats (Toxicity phase). Yes. 2002. Rat. CrI:CD[®] (Sprague-Dawley) IGS BR. Inhalation (gas) 28 days 0, 992, 3033, or 8502 ppm 12 males, 12 females per dose group for main study group 6 hours / day 7 days / wee 12 males, 12 females, air-only exposed. Not applicable All statistical analyses were carried out separately for males and females.</p> <p>Data relating to food consumption was analysed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. The following data types were analysed at each timepoint separately: Rearing and activity counts Bodyweight (FOF) and body temperature Grip strength, landing footsplay and motor activity Bodyweight, using gains over appropriate study periods. Food consumption, over appropriate study periods, using cumulative cage totals. Biochemistry and haematology. Organ weights, absolute and / or adjusted for terminal bodyweight. Pathological findings, for the number of animals with and without each finding. For categorical data, including rearing and activity counts and pathological findings, the proportion of animals was analysed using Fisher's Exact test (Fisher 1973) for each treated group versus the control. For continuous data, Bartlett's test (Bartlett 1937) was first</p>
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<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p>	<p>applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the Control group, incorporating adjustment for multiple comparisons where necessary.</p> <p>The following sequence of statistical tests was used for bodyweight (FOB), body temperature, grip strength, landing footsplay and motor activity, bodyweight, food consumption, organ weight and clinical pathology data.</p> <p>If 75% of the data (across all groups) were the same value, for example c, then a frequency analysis as applied. Treatment groups were compared using a Mantel test for a trend in proportions (Mantel 1963) and also pairwise Fisher's Exact tests (Fisher 1973) for each dose group against the control both for 1) values <c versus values <=c, and for 2) values <=c versus values <c, as applicable.</p> <p>If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Williams' test for a monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead.</p> <p>If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Shirley's test for a monotonic trend (Shirley 1977) was applied. If the H1 test was significant, suggesting that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead.</p> <p>For functional observation battery data and motor activity, pre-treatment values were analysed as detailed above. Data during the treatment phase was analysed using analysis of variance using pre-treatment values as the covariant.</p> <p>For organ weight data, analyses of variance was initially performed using terminal bodyweight as covariate. If the within group relationship between organ weight and bodyweight was significant at the 10% level (Angervall and Carlstrom, 1963), then the treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.</p> <p>Significant differences between Control and treated groups were expressed at the 5% (p<0.05) or 1% (p<0.01) level. Williams test is denoted by '*'; t tests are denoted by '+', and Shirley's test by '±'.</p> <p>992 ppm for females, not established for males (<992 ppm). 3033 ppm for females, <992 ppm for males.</p> <p>The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations.</p>
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<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>Toxicity Phase</p> <p>Salivation was noted in animals receiving 8502 ppm during the treatment period, with lethargy noted on one occasion. Motor activity of males receiving 8502 ppm was reduced throughout the treatment period. Haematocrit and red cell counts were reduced in females exposed to 8502 ppm. Increased kidney weights were noted at 3033 and 8502 ppm, and increased liver weights were noted at 8502 ppm.</p> <p>Histopathological changes included the kidney of male rats (renal cortical tubules with hyaline droplets) with, in all treated males and in females at 3033 and 8502 ppm, as increased incidence of basophilic cortical tubules. Minimal centrilobular hepatocyte hypertrophy of males at 8502 ppm, associated with slightly higher liver weights were noted. In males and females at 8502 ppm and females at 3033 ppm atrophy / disorganisation of the olfactory epithelium of nasal turbinates were noted.</p> <p>Slight effects on general systemic toxicity due to the test substance were apparent amongst male animals receiving 992, 3033 or 8502 ppm. The no effect level of the test substance for the general systemic toxicity to female rats for 28 days inhalation administration was 992 ppm. A no-effect level for male toxicity was not established (<992 ppm).</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd., 2004. 4-week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats Project ID CSS 022. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Repeated Dose Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method / guideline followed Test type</p> <p>GLP Year Species Strain Route of administration Duration of test Doses / concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p> <p>Test conditions</p> <p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p>	<p>Pyrolysis (C5s (CAS No. 8476-55-1, hydrocarbons, C5-rich) are produced from C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1, 3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling point (primary C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in this study.</p> <p>OECD 422 4 week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats (Toxicity phase). Yes. 2002. Rat. CrI:CD® (Sprague-Dawley) IGS BR. Inhalation (gas). 28 days. 0, 98, 302, or 1012 ppm. 12 males, 12 females per dose group for main study group. 6 hours / day. 7 days / week. 12 males, 12 females, air-only exposed. Not applicable. Appropriate statistical analyses were conducted on all parameters.</p> <p>Groups of 12 male and 12 female CD rats were exposed to the test material as a gas daily by inhalation for six hours / day at exposure levels of 0, 98, 302, or 1012 ppm. In this main study (repeated-exposure general toxicity) males and females were exposed for 28 days. During the study, clinical condition, detailed functional observational battery, motor activity, bodyweight, food consumption, haematology, blood chemistry, organ weight and macroscopic and microscopic pathology investigations were undertaken. The study also contained reproductive / developmental toxicity satellite groups (summarized separately).</p> <p>302 ppm for females, not established for males. Not applicable. The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations.</p> <p>Toxicity Phase</p> <p>There were no signs considered reflective of a general systemic effect observed during routine clinical examination or during the</p>
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<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>functional observational battery. Occasional intergroup differences in haematological and biochemical parameters were considered to be unrelated to treatment for the following reasons: no correlation between the sexes, no correlation with other changes, slight magnitude of effects all occurring within expected historical ranges, possible atypical control group results.</p> <p>Histopathological changes were restricted to the liver (minimal centrilobular hepatocyte hypertrophy) of high dose rats, associated with slightly higher liver weights. In addition, in male rats, a higher kidney weight and incidence of cortical tubules with hyaline droplets was apparent in all treated groups. High dose males also showed associated kidney lesions. No kidney pathological changes were apparent in treated females.</p> <p>Slight effects on general systemic toxicity due to the test substance were apparent amongst male animals receiving 98, 302 or 1012 ppm. The no effect level of the test substance for the general systemic toxicity to female rats for 28 days inhalation administration was 302 ppm. A no-effect level for male toxicity was not established.</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd., 2004. 4-week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats Project ID CSS 012. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Toxicity to Reproduction

<p><u>Test Substance</u> Remarks</p>	<p>Isoprene, CAS# 78-79-5 Purity >99%.</p>
<p><u>Method</u> Method/guideline followed Test type GLP Year Species Strain Route of administration Duration of test Concentration levels Sex Exposure period Frequency of treatment Control group and treatment Statistical methods</p>	<p>Other 13-week inhalation study Yes 1994 Rat and mouse F344 rats and B6C3F1 mice Inhalation (vapor) 13 weeks 0, 70, 700, or 7000 ppm 10 male, 10 female per group 6 hours/day 5 days/week 10 male, 10 female, air-only exposed Analysis of incidence of neoplastic and nonneoplastic lesions was performed</p>
<p>Remarks for Test Conditions</p>	<p>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.</p>
<p><u>Results</u> NOAEL</p>	<p>2200 ppm (rats). 220 ppm (mice).</p>
<p><u>Conclusions</u></p>	<p>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).</p> <p>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.</p>

<p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>(1) Reliable with restrictions. Limited reproductive toxicity data obtained as part of a NTP-sponsored subchronic inhalation toxicity study.</p> <p>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.</p>
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Toxicity to Reproduction

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method / guideline followed Test type</p> <p>GLP Year Species Strain Route of administration Duration of test</p> <p>Doses / concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p>	<p>2-methyl-2-butene (CAS number: 513-35-9). The test substance was stable for the duration of all studies performed at the test house</p> <p>OECD 422 4 week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats (Toxicity phase) Yes 2001 / 2002 Rat Cri:CD[®] (Sprague-Dawley) IGS BR Inhalation (gas) Two weeks prior to breeding, during breeding, and continuing through day 19 of gestation. The dams were then allowed to deliver their litters, which were retained until lactation day 4. 0, 580, 2000, or 7000 ppm 12 females per dose group for this satellite study 6 hours / day 7 days / week 12 females, air-only exposed Not applicable All statistical analyses were carried out separately for males and females. Data relating to food consumption was analysed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. The following data types were analysed at each timepoint separately: Rearing and activity counts Bodyweight (FOB) and body temperature Grip strength, landing footsplay and motor activity Bodyweight, using gains over appropriate study periods. Food consumption, over appropriate study periods, using cumulative cage totals. Blood chemistry and haematology. Organ weights, absolute and / or adjusted for terminal bodyweight. Pathological findings, for the number of animals with and without each finding. For categorical data, including rearing and activity counts and pathological findings, the proportion of animals was analysed using Fisher's Exact test (Fisher (1973) for each treated group versus the control. For continuous data, Bartlett's test (Bartlett 1937) was first applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the Control group, incorporating adjustment for multiple comparisons where necessary. The following sequence of statistical tests was used for bodyweight (FOB), body temperature, grip strength, landing foot splay and motor activity, bodyweight, food consumption, organ weight and clinical pathology data.</p>
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<p>Test Conditions</p> <p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p>	<p>If 75% of the data (across all groups) were the same value, for example c, then a frequency analysis was applied. Treatment groups were compared using a Mantel test (for a trend in proportions (Mantel 1963) and also pairwise Fisher's Exact test (Fisher 1973) for each dose group against the control both for 1) values <c versus values >=c, and for 2) values <=c versus values >c, as applicable.</p> <p>If Bartlett's test for variance homogeneity (Bartlett (1937) was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Williams' test for a monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggest that the dose-response as not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead.</p> <p>If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Shirley's test for monotonic trend (Shirley 1977) was applied. If the H1 test was significant, suggesting that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead.</p> <p>For organ weight data, analysis of variance was initially performed using terminal bodyweight as covariate. If the within group relationship between organ weight and bodyweight was significant at the 10% level (Angervall and Carlstrom, 1963), then the treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.</p> <p>Significant differences between Control and treated groups were expressed at the 5% (p>0.05) or 1% (p<0.01) level. Williams test is denoted by '*'; t tests are denoted by '+'; Dunnett's test is denoted by '_*' and Shirley's test by '±'.</p> <p>Satellite groups of 12 female Sprague Dawley rats were exposed to the test material as a gas daily by inhalation for six hours / day at exposure levels of 0, 580, 2000, or 7000 ppm. The study design included a main study for repeated dose toxicity end points (summarized separately) and reproductive / developmental toxicity satellite groups of 12 females per exposure level. The reproductive / developmental toxicity satellite groups were exposed for two weeks prior to breeding, during breeding and continuing through day 19 of gestation. Males from the main study were used to breed these females. The dams were allowed to deliver their litters, which were retained until lactation Day 4. During the study clinical condition, bodyweight, food consumption, oestrus cycles, mating performance, litter data, organ weights and macroscopic pathology were undertaken.</p> <p>7000 ppm. Not applicable. The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations.</p>
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<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>Reproductive Phase Exposure to female rats for 2 weeks prior to pairing, and up to Day 19 of gestation, did not produce any evidence of any reproduction or developmental toxicity. The oestrus cycle was unaffected by exposure, and mating performance, fertility indices and gestation length were similar in all groups. There were no adverse effects upon survival or growth of the offspring in utero or up to Day 4 of lactation.</p> <p>The no effect level for reproduction / developmental toxicity was 7000</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd., 2004. 4-week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats Project ID CSS 002. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Toxicity to Reproduction

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method / guideline followed Test type</p> <p>GLP Year Species Strain Route of administration Duration of test</p> <p>Doses concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pryolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffinic / Olefin / Naphthene / Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (75%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9.</p> <p>The test substance was considered to be stable for the duration of the study.</p> <p>OECD 422 4 week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats (Reproductive phase).</p> <p>Yes 2002 Rat CrI:CD[®] (Sprague-Dawley) IGS BR. Inhalation (gas) Two weeks prior to mating, during mating, and continuing through day 19 of gestation. The dams were then allowed to deliver their litters, which were retained until lactation day 4. 0, 992, 3033, or 8502 ppm 12 females per dose group 6 hours / day 7 days / week 12 females, air-only exposed Not applicable All statistical analyses were carried out separately for males and females. Data relating to food consumption was analysed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. The following data types were analysed at each timepoint separately: Bodyweight, using gains over appropriate study periods. Food consumption, over appropriate study periods, sing cumulative cage totals. Organ weights, absolute and / or adjusted for terminal bodyweight. For continuous data, Bartlett's test (Bartlett (1937) was first applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the Control group, incorporating adjustment for multiple comparisons where necessary. The following sequence of statistical tests was used for bodyweight, food consumption, organ weight and litter data. If 75% of the data (across all groups) were the same value, for</p>
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<p>Test Conditions</p> <p>Results NOAEL (NOEL) LOAEL (LOEL) Remarks</p>	<p>example c, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions (Mantel 1963) and also pairwise Fisher's Exact tests (Fisher 1973) for each dose group against the control both for 1) values <c versus values >=c, and for 2) values <=c versus values <c, as applicable.</p> <p>If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Williams' test for monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead.</p> <p>If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Shirley's test for a monotonic trend (Shirley 1977) was applied. If the H1 test was significant, suggesting that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead.</p> <p>For organ weight data, analysis of variance was initially performed using terminal bodyweight as covariate. If the within group relationship between organ weight and bodyweight was significant at the 10% level (Angervall and Carlstrom, 1963), then the treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.</p> <p>Significant differences between Control and treated groups were expressed at the 5% (p<0.05) or 1% (p<0.01) level. Williams test is denoted by '*'; t tests are denoted by '+', and Shirley's test by '±'.</p> <p>Groups of 12 female Sprague Dawley rats were exposed to the test material as a gas daily by inhalation for six hours / day at exposure levels of 0, 992, 3033 or 8502 ppm. The study design included a main study for repeated dose toxicity end points (summarized separately) and reproductive / developmental toxicity satellite groups of 12 females per exposure level. The reproductive / developmental toxicity satellite groups were exposed for two weeks prior to mating, during mating and continuing through day 19 of gestation. Males from the main study were used to breed these females. The dams were allowed to deliver their litters, which were retained until lactation day 4. During the study clinical condition, bodyweight, food consumption, oestrous cycles, mating performance, litter data, organ weights and macroscopic pathology were undertaken.</p> <p>8502 ppm. Not applicable. The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations.</p> <p>Reproductive Phase Exposure to male and female rats for 2 weeks prior to pairing, and for female rats up to day 19 of gestation, did not produce</p>
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<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>any evidence of any reproduction or developmental toxicity. The oestrous cycle was unaffected by exposure, and mating performance, fertility indices and gestation length were similar in all groups. There were no adverse effects upon survival or growth of the offspring <i>in utero</i> or up to day 4 of lactation.</p> <p>The no effect level for reproductive / developmental toxicity was 8502 ppm.</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd., 2004. 4-week general toxicity and reproductive / developmental toxicity screening test by inhalation 3exposure to rats Project ID CSS 022. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Toxicity to Reproduction

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method / guideline followed Test type</p> <p>GLP Year Species Strain Route of administration Duration of test</p> <p>Doses / concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p> <p>Test conditions</p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced from C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1, 3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl 1-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling point (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8.</p> <p>The three major components analysed were shown to be stable for the duration of testing in this study.</p> <p>OECD 422 4 week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats (Reproductive phase).</p> <p>Yes 2002 Rat CrI:CD[®] (Sprague-Dawley) IGS BR Inhalation (gas) Two weeks prior to breeding, during breeding, and continuing through day 19 of gestation. The dams were then allowed to deliver their litters, which were retained until lactation day 4. 0, 98, 302 or 1012 ppm 12 females per dose group for this satellite study 6 hours / day 7 days / week 12 females, air-only exposed Not applicable Appropriate statistical analyses were conducted on all parameters Satellite groups of 12 female Sprague Dawley rats were exposed to the test material as a gas daily by inhalation for six hours / day at exposure levels of 0, 98, 302, 94 102 ppm. The study design included a main study for repeated dose toxicity end points (summarized separately) and reproductive / developmental toxicity satellite groups of 12 females per exposure level. The reproductive / developmental toxicity satellite groups were exposed for two weeks prior to breeding, during breeding and continuing through day 19 of gestation. Males from the main study were used to breed these females. The dams were allowed to deliver their litters, which were retained until lactation day 4. During the study clinical condition, bodyweight, food consumption, oestrus cycles, mating performance, litter data, organ weights and macroscopic pathology was undertaken.</p>
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<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>1012 ppm. Not applicable. The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations.</p> <p>Reproductive Phase Exposure to female rats for 2 weeks prior to pairing, and up to day 19 of gestation, did not produce any evidence of any reproduction or developmental toxicity. The oestrous cycle was unaffected by exposure, and mating performance fertility indices and gestation length were similar in all groups. There were no adverse effects upon survival or growth of the offspring <i>in utero</i> or up to day 4 of lactation.</p> <p>The no effect level for reproduction / developmental toxicity was 1012 ppm.</p> <p>(1) Reliable without restrictions</p> <p>Huntingdon Life Sciences Ltd., 2004. 4-week general toxicity and reproduction / development toxicity screening test by inhalation exposure to rats Project ID CSS 012. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Developmental Toxicity/Teratogenicity

<u>Test Substance</u>	Isoprene, CAS# 78-79-5
Remarks	Purity >99.7%.
<u>Method</u>	
Method/guideline followed	OECD 414
Test type	Developmental toxicity (teratogenicity) study
GLP	Yes
Year	1989
Species	Rat and mouse
Strain	Sprague-Dawley (rat) and CD-1/Swiss (mouse)
Route of administration	Inhalation (vapor)
Concentration levels	0, 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 day 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.
<u>Results</u>	
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-1) 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 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

<p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>intrauterine death was present at any exposure level. There was no significant increase in the incidence of fetal malformations or ossifications, 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. Increased incidences of variations (supernumerary ribs) were observed in the exposed groups, although this skeletal variation is generally considered a secondary effect of maternal toxicity or stress and it's significance is unclear. The incidence of supernumerary ribs (percent of fetuses examined) was 20.1, 23.8, 33.6, and 40.3% at 0, 280, 1400, and 7000 ppm.</p> <p>Pregnant Sprague-Dawley rats and their offspring exhibited no significant toxic effects of isoprene at any exposure level in this study. Swiss (CD-1) 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.</p> <p>(1) Reliable without restrictions. NTP-sponsored study.</p> <p>National Toxicology Program (1989). Inhalation Developmental Toxicology Studies: Teratology Study of Isoprene in Mice and Rats. TER88045; NTIS#DE89008095.</p>
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AQUATIC TOXICITY ROBUST SUMMARIES**Fish Acute Toxicity**

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed GLP Year Test type Species Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p> <p><u>Results</u></p>	<p>Isoprene (CAS No. 78-79-5) also known as 1,3-butadiene,2-methyl The test substance was stable for the duration of all the studies performed by the test house.</p> <p>OECD Guide-line 203 and EC directive 92/96 C1 Yes 2002 Semistatic <i>Oncorhynchus mykiss</i> (fresh water fish) 96 hours No Yes Groups of ten juvenile fish were exposed for 96 hours to Isoprene, prepared in diluent water (dechlorinated tap water, ca. 150-200 mg/l as CaCO₃) at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. Because the test substance was known to be volatile, the test was conducted in completely filled and sealed vessels. At each concentration, the test medium was prepared from an aqueous preparation which was made using techniques designed for generating a water accommodated fraction (WAF); the test substance was injected through a silicone bung into a glass vessel containing diluent water and was stirred for approximately 24 hours before aliquots were removed and used to fill duplicate test vessels at each concentration.</p> <p>The exposure levels were monitored by measuring the concentrations of Isoprene in samples of the test media using a GC method of analysis. The measured concentrations of Isoprene ranged between 42% and 105% of their nominal values in samples of freshly prepared media and between 44 and 84% % of their nominal values in samples of expired (24-hour-old) media (between 68 and 106% of their starting values). Based on a geometric mean, the overall mean measured levels of Isoprene were 1.68, 3.57, 6.71, 15.0 and 28.7 and 28.7 mg/l. Observations of the fish were made after 0.25, 2, 4, 24, 48, 72 and 96 hours of exposure. LC50 values were estimated by a computer program (Stephan:1977,1982) using the number of fish exposed and the number dead at each measured concentration.</p> <p>After 96 hours, the highest measured concentration at which no mortality had occurred was 3.57 mg/l and the lowest at which there was 100% mortality was 15.0 mg/l. Treatment-related effects were exhibited at 6.71 mg/l and higher concentrations. Based on these findings the following values have been</p>
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<p><u>Conclusions</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>estimated: 96-hour LC50 value = 7.43 mg/l (95% confidence limits of 6.71 and 15.0 mg/l)</p> <p>NOEC = 3.57 mg/l (measured concentration) LC50 = 7.43 mg/l (measured concentration)</p> <p>(1) Valid without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 032. Huntingdon Life Sciences Ltd., Cambridgeshire, England</p>
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Fish Acute Toxicity

<p><u>Test Substance</u></p> <p>Remarks</p> <p><u>Method</u></p> <p>Method/guideline followed GLP Year Test type Species Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p> <p><u>Results</u></p>	<p>2-methyl-2-butene (CAS No. 513-35-9) also known as 2-methyl, 2-butene</p> <p>The test substance was stable for the duration of all the studies performed by the test house.</p> <p>OECD Guide-line 203 and EC directive 92/96 C1 Yes 2002 Semistatic <i>Oncorhynchus mykiss</i> (Rainbow trout; fresh water fish) 96 hours No Yes</p> <p>The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of 2-methyl-2-butene. At each concentration, the test medium was prepared by stirring the test substance in a sealed vessel for approximately 24 hours. After being allowed to stand for at least 30 minutes to obtain an equilibrium concentration of 2-methyl-2-butene, aliquots of medium were removed from the middle of the vessel and used to fill the test vessels at each concentration.</p> <p>Groups of ten juvenile fish were exposed for 96 hours to a control solution (dechlorinated tap water, ca. 150-200 mg/l as CaCO₃) or to 2-methyl-2-butene at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of 2-methyl-2-butene in samples of the test media using a GLC method of analysis.</p> <p>The measured concentrations of 2-methyl-2-butene ranged between 33% and 89% of their nominal values in samples of freshly prepared media and between 33 and 99% of their nominal values in samples of expired (24-hour-old) media (between 93 and 116% of their starting values). Based on an arithmetic mean, the overall mean measured levels of 2-methyl-2-butene were 1.67, 2.93, 5.33, 8.51 and 25.9 mg/l.</p> <p>Observations of the fish were made after 0.25, 2, 4, 24, 48, 72 and 96 hours of exposure.</p> <p>LC₅₀ values were estimated by non-linear interpolation between the two concentrations which bracket the 50% effect level using a computer program (Stephan:1977, 1982); the program uses the number of fish exposed and the number dead at each measured concentration.</p> <p>After 96 hours, the highest measured concentration at which no mortality had occurred was 2.93 mg/l and the lowest at which there was 100% mortality was 8.51 mg/l. Treatment-related effects were exhibited at 5.33 mg/l and higher concentrations.</p>
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<p><u><i>Conclusions</i></u></p> <p><u><i>Data Quality</i></u></p> <p><u><i>Reference</i></u></p>	<p>Based on these findings the following values have been estimated: 96-hour LC₅₀ value = 4.99 mg/l (95% confidence limits of 2.93 and 8.51 mg/l).</p> <p>NOEC = 2.93 mg/l (measured concentration) LC₅₀ = 4.99 mg/l (measured concentration)</p> <p>(1) Valid without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 032. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Fish Acute Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed GLP Year Test type Species Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p> <p><u>Results</u></p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content.</p> <p>The Paraffin/Olefin/Naphthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%).</p> <p>Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9.</p> <p>The test substance was considered to be stable for the duration of the study.</p> <p>OECD Guide-line 203 and EC Directive 92/96 C1 Yes 2002 Semistatic <i>Oncorhynchus mykiss</i> (Rainbow trout; fresh water fish) 96 hours No Yes</p> <p>The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Hydrotreated C5s. At each concentration, the test medium was prepared by stirring the test substance in a sealed vessel for between approximately 20 and 23 hours. After being allowed to stand for at least 60 minutes to obtain an equilibrium concentration of Hydrotreated C5s, aliquots of medium were removed from the middle of the vessel and used to fill the test vessels at each concentration.</p> <p>Groups of ten juvenile fish were exposed for 96 hours to a control solution (dechlorinated tap water, ca. 150-200 mg/l as CaCO₃) or to Hydrotreated C5s at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of Hydrotreated C5s in samples of the test media using a GC method of analysis.</p> <p>Observations of the fish were made after 0.25, 2, 4, 24, 48, 72 and 96 hours of exposure.</p> <p>LL₅₀/LC₅₀ values were estimated by non-linear interpolation between the two loading rates/concentrations which bracket the 50% effect level using a computer program (Stephan:1977, 1982); the program uses the number of fish exposed and the number dead at each nominal loading rate/measured concentration.</p> <p>The measured concentrations of Hydrotreated C5s ranged</p>
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	<p>between 36% and 79% of their nominal values in samples of freshly prepared media and between 27 and 67% of their nominal values in samples of expired (24-hour-old) media (between 62 and 92% of their starting values). Based on a geometric mean, the overall mean measured levels of Hydrotreated C5s were 1.12, 2.79, 5.33, 10.3 and 19.6 mg/l.</p> <p>After 96 hours, the highest nominal loading rate at which $\leq 10\%$ mortality had occurred was 4.70 mg/l and the lowest at which there was 100% mortality was 22.7 mg/l; 2.79 and 10.3 mg/l in terms of mean measured Hydrotreated C5s concentrations.</p> <p>Treatment-related effects were exhibited at nominal loading rates of 4.70 mg/l and higher rates (2.79 mg/l, measured).</p> <p>Based on these findings the following values have been estimated: 96-hour LL_{50} value = 10.3 mg/l (95% confidence limits of 4.7 and 22.7 mg/l) 96-hour LC_{50} value = 5.33 mg/l (95% confidence limits of 2.79 and 10.3 mg/l).</p> <p><u>Conclusions</u></p> <p>96-hour LL_{50} = 10.3 mg/l (loading rate) 96-hour LC_{50} = 5.33 mg/l (measured concentration)</p> <p><u>Data Quality</u></p> <p>(1) Valid without restrictions</p> <p><u>Reference</u></p> <p>Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 025. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Fish Acute Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed GLP Year Test type Species Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p> <p><u>Results</u></p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in the study.</p> <p>OECD Guide-line 203 and EC Directive 92/96 C1 Yes 2002 Semistatic <i>Oncorhynchus mykiss</i> (Rainbow trout; fresh water fish) 96 hours No Yes</p> <p>The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Pyrolysis C5s. At each concentration, the test medium was prepared by stirring the test substance in a sealed vessel for between approximately 21 and 22 hours. After being allowed to stand for at least 60 minutes to obtain an equilibrium concentration of Pyrolysis C5s, aliquots of medium were removed from the middle of the vessel and used to fill the test vessels at each concentration.</p> <p>Groups of ten juvenile fish were exposed for 96 hours to a control solution (dechlorinated tap water, ca. 150-200 mg/l as CaCO₃) or to Pyrolysis C5s at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of Pyrolysis C5s in samples of the test media using a HPLC method of analysis.</p> <p>Observations of the fish were made after 0.25, 2, 4, 24, 48, 72 and 96 hours of exposure.</p> <p>LL₅₀/LC₅₀ values were estimated by non-linear interpolation between the two loading rates/concentrations which bracket the 50% effect level using a computer program (Stephan:1977, 1982); the program uses the number of fish exposed and the number dead at each nominal loading rate/measured concentration.</p> <p>The measured concentrations of Pyrolysis C5s ranged between 37% and 91% of their nominal values in samples of freshly prepared media and between 24 and 73% of their nominal values in samples of expired (24-hour-old) media (between 62</p>
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	<p>and 112% of their starting values). Based on an arithmetic mean, the overall mean measured levels of Pyrolysis C5s were 1.42, 2.49, 6.40, 12.8 and 27.0 mg/l.</p> <p>After 96 hours, the highest nominal loading rate at which $\leq 10\%$ mortality had occurred was 10.3 mg/l and the lowest at which there was 100% mortality was 22.7 mg/l; 6.40 and 12.8 mg/l in terms of mean measured Pyrolysis C5s concentrations.</p> <p>Treatment-related effects were exhibited at nominal loading rates of 4.70 mg/l and higher rates (2.49 mg/l, measured).</p> <p>Based on these findings the following values have been estimated: 96-hour LL_{50} value = 14.1 mg/l (95% confidence limits of 10.3 and 22.7 mg/l) 96-hour LC_{50} value = 8.41 mg/l (95% confidence limits of 6.40 and 12.8 mg/l).</p> <p><u>Conclusions</u></p> <p>96-hour LL_{50} = 14.1 mg/l (loadig rate) 96-hour LC_{50} = 8.41 mg/l (measured concentration)</p> <p><u>Data Quality</u></p> <p>(1) Valid without restrictions</p> <p><u>Reference</u></p> <p>Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 015. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Fish Acute Toxicity

Test Substance	CAS No. 109-66-0; 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 (<i>Oncorhynchus mykiss</i>)
Analytical Monitoring	Yes
Exposure Period	96 hour
Statistical Method	Trimmed Spearman Karber Method.
Test Conditions	<p>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 re-dosing. Each test vessel contained 5 fish. Three replicates were prepared per treatment, two with fish and one for sampling purposes.</p> <p>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.</p> <p>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.</p> <p>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.</p>
<ul style="list-style-type: none"> Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, weight, loading. 	

<p>Results</p> <p>Units/Value:</p> <ul style="list-style-type: none"> Note: Deviations from protocol or guideline, analytical method, biological observations, control survival. 	<p>96 hour LC50 = 4.26mg/L (95% CI 3.6 to 5.04mg/L) based upon measured values of old and new solutions.</p> <p>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.</p> <table border="1" data-bbox="690 541 1169 787"> <thead> <tr> <th><u>Measured Conc. (mg/L)</u></th> <th><u>Fish Total Mortality (@96 hrs)*</u></th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>0</td> </tr> <tr> <td>0.635</td> <td>0</td> </tr> <tr> <td>1.02</td> <td>0</td> </tr> <tr> <td>2.21</td> <td>0</td> </tr> <tr> <td>5.81</td> <td>7</td> </tr> <tr> <td>7.03</td> <td>10</td> </tr> </tbody> </table> <p>* 10 fish added at test initiation</p>	<u>Measured Conc. (mg/L)</u>	<u>Fish Total Mortality (@96 hrs)*</u>	Control	0	0.635	0	1.02	0	2.21	0	5.81	7	7.03	10
<u>Measured Conc. (mg/L)</u>	<u>Fish Total Mortality (@96 hrs)*</u>														
Control	0														
0.635	0														
1.02	0														
2.21	0														
5.81	7														
7.03	10														
<p>Conclusion</p>	<p>96 hour LC50 = 4.26mg/L (95% CI 3.6 to 5.04mg/L) based upon measured values of old and new solutions.</p>														
<p>Reliability</p>	<p>(1) Reliable without restriction</p>														
<p>Reference</p>	<p>Exxon Biomedical Sciences, Inc. 1997. Acute Fish Toxicity Test with Rainbow Trout. Study #157558.</p>														

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 (<i>Oncorhynchus mykiss</i>)
Analytical Monitoring	Yes
Exposure Period	96-hour
Statistical Method	Trimmed Spearman-Kärber Method (Hamilton, M.A. <i>et al.</i> 1977. Trimmed Spearman-Kärber Method for Estimating Median Lethal Concentration in Toxicity Bioassays. <i>Environ. Sci. Technol.</i> 11:714-719.)
Test Conditions	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 $\leq 10\%$. 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.
<ul style="list-style-type: none"> Note: Test material loading preparation, vessel type, volume, replication, water quality parameters, environmental conditions, and test organism supplier, age, size, weight, and loading. 	<p>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).</p>

<p>Test Conditions (cont.)</p> <p>Note: Test material loading preparation, vessel type, volume, replication, water quality parameters, environmental conditions, and test organism supplier, age, size, weight, and loading.</p> <p>Results</p> <p>Units/Value:</p> <ul style="list-style-type: none"> Note: Deviations from protocol or guideline, analytical method, biological observations, control survival. <p>Conclusion</p> <p>Reliability</p> <p>Reference</p>	<p>Test temperature was 16C (sd = 0.04). Lighting was 623 to 629 Lux with a 16-hr 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.</p> <p>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.</p> <p>96-hour LL50 = 12.8 mg/L (95% CI 10.7 to 15.3 mg/L) based upon loading rates.</p> <table border="1" data-bbox="711 758 1117 1003"> <thead> <tr> <th>Loading Rate (mg/L)</th> <th>Fish Total Mortality (@96 hrs)*</th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>0</td> </tr> <tr> <td>6.25</td> <td>0</td> </tr> <tr> <td>12.5</td> <td>7</td> </tr> <tr> <td>25</td> <td>15</td> </tr> <tr> <td>50</td> <td>15</td> </tr> <tr> <td>100</td> <td>15</td> </tr> </tbody> </table> <p>* 15 fish added at test initiation</p> <p>96-hour LL50 = 12.8 mg/L (95% CI 10.7 to 15.3 mg/L) based upon loading rates.</p> <p>(1) Reliable without restriction</p> <p>Exxon Biomedical Sciences, Inc. 1996. Fish, Acute Toxicity Test. Study #119058. Exxon Biomedical Sciences, Inc., East Millstone, NJ, USA.</p>	Loading Rate (mg/L)	Fish Total Mortality (@96 hrs)*	Control	0	6.25	0	12.5	7	25	15	50	15	100	15
Loading Rate (mg/L)	Fish Total Mortality (@96 hrs)*														
Control	0														
6.25	0														
12.5	7														
25	15														
50	15														
100	15														

Daphnid Acute Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed GLP Year Test type Species Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p> <p><u>Results</u></p>	<p>Isoprene (CAS No. 78-79-5) also known as 1,3-butadiene,2-methyl The test substance was stable for the duration of all the studies performed by the test house.</p> <p>OECD Guide-line 202 and EC Directive 92/96 C2 Yes 2002 Static <i>Daphnia magna</i> (Crustacea) 48 hours No Yes Groups of twenty <i>Daphnia</i>, less than 24 hours old, were exposed for 48 hours to Isoprene, prepared in Elendt M4 medium at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. Because the test substance was known to be volatile, the test was conducted in completely filled and sealed vessels. The test media were prepared, either directly or by dilution, from an aqueous preparation which was made using techniques designed for generating a water accommodated fraction (WAF); the test substance was injected through a silicone septum into an amber glass vessel containing Elendt M4 medium and was stirred for approximately 22 hours before aliquots were removed and used to provide the test media.</p> <p>The exposure levels were monitored by measuring the concentrations of Isoprene in samples of the test media using a GC method of analysis.</p> <p>Observations of the <i>Daphnia</i> in each control and test vessel were made after 24 and 48 hours. EC50 values were calculated using a computer program (Stephan's: 1977,1982) which uses the number of <i>Daphnia</i> exposed and the number immobile at each nominal and measured concentration.</p> <p>The "no observed effect concentration" (NOEC) was derived by direct inspection of the data on the immobility of the animals. An incidence of more than 10% is considered to be significant.</p> <p>Although lower than intended, the measured concentrations of Isoprene at the start (between 30 and 48% of their nominal values) were adequately maintained during the test, giving measured levels of between 30 and 51% of nominal after 48 hours. Based on an arithmetic mean, the overall mean measured levels of Isoprene were 0.648, 1.55, 3.52, 9.47 and 25.4 mg/l. After 48 hours, the lowest measured concentration resulting in 100% immobility was 9.47 mg/l and the highest measured concentration at which no immobilisation occurred was 3.52</p>
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	<p>mg/l.</p> <p><u>Conclusions</u></p> <p>48-hour EC50 value = 5.77 mg/l (95% confidence limits of 3.52 and 9.47 mg/l)(measured concentration) NOEC = 3.52 mg/l (measured concentration)</p> <p><u>Data Quality</u></p> <p>(1) Valid without restrictions</p> <p><u>Reference</u></p> <p>Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to <i>Daphnia Magna</i>. Project ID CSS 033 Huntingdon Life Sciences Ltd., Cambridgeshire, England</p>
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Daphnid Acute Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed GLP Year Test type Species Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p> <p><u>Results</u></p>	<p>2-methyl-2-butene (CAS No. 513-35-9) The test substance was stable for the duration of all the studies performed by the test house.</p> <p>OECD Guide-line 202 and EC Directive 92.96 C2 Yes 2002 Static <i>Daphnia magna</i> (Crustacea) 48 hours No Yes The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of 2-methyl-2-butene. The test media were prepared, either directly or by dilution, from an aqueous preparation in which the test substance was stirred in a sealed vessel in the dark for approximately 24 hours. After being allowed to stand for approximately 30 minutes to obtain an equilibrium concentration of 2-methyl-2-butene, aliquots of medium were removed from the middle of the vessel and used to fill replicate vessels at each concentration.</p> <p>Groups of twenty <i>Daphnia</i>, less than 24 hours old, were exposed for 48 hours to 2-methyl-2-butene, prepared in Elendt M4 medium at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of 2-methyl-2-butene in samples of the test media using a GLC method of analysis.</p> <p>Observations of the <i>Daphnia</i> in each control and test vessel were made after 24 and 48 hours.</p> <p>EC₅₀ values were estimated either by the moving average method or by non-linear interpolation between the two concentrations which bracket the 50% effect level using a computer program (Stephan:1977, 1982); the program uses the number of <i>Daphnia</i> exposed and the number immobile at each nominal and measured concentration.</p> <p>The "no observed effect concentration" (NOEC) was derived by direct inspection of the data on the immobility of the animals. An incidence of more than 10% is considered to be significant.</p> <p>Although lower than intended, the mean measured concentrations of 2-methyl-2-butene at the start (between 30 and 49% of their nominal values) were adequately maintained during the test, giving measured levels of between 28 and 46% of nominal after 48 hours. Based on an arithmetic mean, the overall mean measured levels of isoprene were 0.691, 1.74, 2.95, 6.63 and 23.6 mg/l.</p>
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	<p>After 48 hours, the lowest measured concentration resulting in 100% immobility was 6.63 mg/l and the highest measured concentration at which immobilisation was \leq 10% was 1.74 mg/l.</p> <p><u>Conclusions</u></p> <p>48-hour EC₅₀ value = 3.84 mg/l (95% confidence limits of 3.01 and 4.80 mg/l; measured concentration) NOEC = 1.74 mg/l (measured concentration)</p> <p><u>Data Quality</u></p> <p>(1) Valid without restrictions</p> <p><u>Reference</u></p> <p>Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to <i>Daphnia Magna</i>. Project ID CSS 033. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Daphnid Acute Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed GLP Year Test type Species Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p> <p><u>Results</u></p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content.</p> <p>The Paraffin/Olefin/Naphthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%).</p> <p>Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9.</p> <p>The test substance was considered to be stable for the duration of the study.</p> <p>OECD Guide-line 202 and EC Directive 92/96 C2 Yes 2002-2003 Static <i>Daphnia magna</i> (Crustacea) 48 hours No Yes</p> <p>The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Hydrotreated C5s. At each concentration, the test medium was prepared from an aqueous preparation in which the test substance was stirred in a sealed vessel in the dark for approximately 20.5 hours. After being allowed to stand for approximately 1.5 hours to obtain an equilibrium concentration of Hydrotreated C5s, aliquots of medium were removed from the middle of the vessel and used to fill replicate vessels at each concentration.</p> <p>Groups of twenty <i>Daphnia</i>, less than 24 hours old, were exposed for 48 hours to Hydrotreated C5s, prepared in Elendt M4 medium at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of Hydrotreated C5s in samples of the test media using a GLC method of analysis.</p> <p>Observations of the <i>Daphnia</i> in each control and test vessel were made after 24 and 48 hours.</p> <p>EL₅₀/EC₅₀ values were estimated by non-linear interpolation between the two loading rates/concentrations which bracket the 50% effect level using a computer program (Stephan:1977, 1982); the program uses the number of <i>Daphnia</i> exposed and the number immobile at each nominal loading rate and measured concentration.</p> <p>Although lower than intended, the mean measured</p>
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<p><u>Conclusions</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>concentrations of Hydrotreated C5s at the start (between 14 and 31% of their nominal values) were adequately maintained during the test, giving measured levels of between 18 and 40% of nominal after 48 hours. Based on a geometric mean, the overall mean measured levels of Hydrotreated C5s were 0.338, 0.783, 3.60, 6.77 and 15.3 mg/l.</p> <p>After 48 hours, the highest loading rate at which no immobilisation occurred was 4.70 mg/l and the lowest loading rate resulting in 100% immobility was 22.7 mg/l; 0.783 and 6.77 mg/l in terms of mean measured Hydrotreated C5s concentrations.</p> <p>48-hour EL₅₀ value = 9.34 mg/l (95% confidence limits of 4.70 and 22.7 mg/l; nominal loading rate) 48-hour EC₅₀ value = 2.98 mg/l (95% confidence limits of 0.783 and 6.77 mg/l; measured concentration)</p> <p>48-hour EL₅₀ value = 9.34 mg/l (nominal loading rate) 48-hour EC₅₀ value = 2.98 mg/l (measured concentration)</p> <p>(1) Valid without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2003. Acute Toxicity to <i>Daphnia magna</i>. Project ID CSS 024. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Daphnid Acute Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed GLP Year Test type Species Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p> <p><u>Results</u></p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in the study.</p> <p>OECD Guide-line 202 and EC Directive 92/96 C2 Yes 2002-2003 Static <i>Daphnia magna</i> (Crustacea) 48 hours No Yes</p> <p>The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Pyrolysis C5s. At each concentration, the test medium was prepared from an aqueous preparation in which the test substance was stirred in a sealed vessel in the dark for approximately 23 hours. After being allowed to stand for approximately 1.5 hours to obtain an equilibrium concentration of Pyrolysis C5s, aliquots of medium were removed from the middle of the vessel and used to fill replicate vessels at each concentration.</p> <p>Groups of twenty <i>Daphnia</i>, less than 24 hours old, were exposed for 48 hours to Pyrolysis C5s, prepared in Elenit M4 medium at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of Pyrolysis C5s in samples of the test media using an HPLC method of analysis.</p> <p>Observations of the <i>Daphnia</i> in each control and test vessel were made after 24 and 48 hours.</p> <p>EL₅₀/EC₅₀ values were estimated by non-linear interpolation between the two loading rates/concentrations which bracket the 50% effect level using a computer program (Stephan:1977, 1982); the program uses the number of <i>Daphnia</i> exposed and the number immobile at each nominal loading rate and measured concentration.</p> <p>Although lower than intended, the mean measured concentrations of Pyrolysis C5s at the start (between 54 and 67% of their nominal values) were adequately maintained</p>
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	<p>during the test, giving measured levels of between 55 and 73% of nominal after 48 hours. Based on an arithmetic mean, the overall mean measured levels of Pyrolysis C5s were 1.41, 3.23, 6.83, 15.6 and 27.2 mg/l.</p> <p>After 48 hours, the highest loading rate at which no immobilisation occurred was 4.70 mg/l and the lowest loading rate resulting in 100% immobility was 10.3 mg/l; 3.23 and 6.83 mg/l in terms of mean measured Pyrolysis C5s concentrations.</p> <p>48-hour EL_{50} value = 6.96 mg/l (95% confidence limits of 4.70 and 10.3 mg/l; nominal loading rate) 48-hour EC_{50} value = 4.70 mg/l (95% confidence limits of 3.23 and 6.83 mg/l; measured concentration)</p> <p><u>Conclusions</u></p> <p>48-hour EL_{50} = 6.96 mg/l (nominal loading rate) 48-hour EC_{50} = 4.70 mg/l (measured concentration)</p> <p><u>Data Quality</u></p> <p>(1) Valid without restrictions</p> <p><u>Reference</u></p> <p>Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to <i>Daphnia Magna</i>. Project ID CSS 014. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Alga Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed GLP Year Endpoint Species</p> <p>Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p>	<p>Isoprene (CAS No. 78-79-5) also known as 1,3-butadiene,2-methyl The test substance was stable for the duration of all the studies performed by the test house.</p> <p>OECD Guide-line 201 and EC Directive 92/69 C3 Yes 2002 Growth rate Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) (Algae) 96 hours No Yes</p> <p>Triplicate algal cultures, with an initial cell density of 1 x 10⁴/ml, were exposed to Isoprene at nominal concentrations of 4.27, 9.39, 20.7, 45.5 and 100 mg/l. Because the test substance was known to be volatile, the test was conducted in completely filled and sealed vessels. The test media were prepared, either directly or by dilution, from an aqueous preparation which was made using techniques designed for generating a water accommodated fraction (WAF); the test substance was injected through a silicone septum into an amber glass vessel containing algal medium and was stirred for approximately 21 hours before aliquots were removed and used to provide the test media. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 23.4 to 24.0°C for 96 hours.</p> <p>The exposure levels were monitored by measuring the concentrations of Isoprene in samples of the test media using a GC method of analysis.</p> <p>Cell numbers were measured daily to monitor growth, and the test results are expressed in terms of the area under the growth curve and growth rate.</p> <p>The area under the growth curve is taken to be an index of growth and is calculated mathematically. The E_bC₅₀ (“x”h) is the median effect concentration for inhibition of growth based on a comparison of areas under the growth curves after “x” hours. The E_bC₅₀ was calculated by a computer program (Stephan:1977, 1982) using percentage effect and the nominal and measured test concentration in test samples. The “no observed effect concentrations” (NOEC) was determined using Dunnett’s multicomparison test to compare the percentage inhibition in the test group with that for the control cultures (Dunnett:1955, 1964).</p>
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Alga Toxicity

<p><u>Test Substance</u></p> <p>Remarks</p> <p><u>Method</u></p> <p>Method/guideline followed</p> <p>GLP</p> <p>Year</p> <p>Endpoint</p> <p>Species</p> <p>Exposure period</p> <p>Limit test</p> <p>Analytical monitoring</p> <p>Study Design</p> <p>Evaluation of data</p>	<p>2-methyl-2-butene (CAS No. 513-35-9) also known as 2-methyl, 2-butene.</p> <p>The test substance was stable for the duration of all the studies performed by the test house.</p> <p>OECD Guide-line 201, EC Directive 92/69 C3, US EPA TSCA 797.1050 & 797.1060</p> <p>Yes</p> <p>2003</p> <p>Growth rate</p> <p><i>Pseudokirchneriella subcapitata</i> (formerly <i>Selenastrum capricornutum</i>) (Algae)</p> <p>96 hours</p> <p>No</p> <p>Yes</p> <p>The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of 2-methyl-2-butene. The test media were prepared, either directly or by dilution, from an aqueous preparation in which the test substance was stirred in a sealed vessel for approximately 23 hours in the dark. After being allowed to stand for at least one hour to obtain an equilibrium concentration of 2-methyl-2-butene, aliquots of medium were removed from the middle of the vessel and after dilution and inoculated with algal cells, was used to fill the test vessels. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 22.3 to 23.4°C for 96 hours. Replicate algal cultures, with an initial cell density of 1×10^4/ml, were exposed to 2-methyl-2-butene at nominal concentrations of 3.20, 7.04, 15.5, 34.1 and 75 mg/l.</p> <p>The exposure levels were monitored by measuring the concentrations of isoprene in samples of the test media using a GLC method of analysis.</p> <p>Cell densities were measured daily to monitor growth, and the test results are expressed in terms of the area under the growth curve and growth rate.</p> <p>The area under the growth curve and the average specific growth rate are taken to be an index of growth and are calculated mathematically.</p> <p>The E_bC_{50} ("x" h) is the median effect concentration for inhibition of growth based on a comparison of areas under the growth curves after "x" hours. The E_bC_{50} was calculated using the moving average method of a computer program (Stephan:1977, 1982) which uses percentage effect and the nominal and measured test concentration in test samples.</p> <p>The E_rC_{50} ("x" - "y" h) is the median effect concentration for inhibition of growth based on a comparison of growth rates</p>
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<p><u>Results</u></p> <p>Observations</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>from "x" to "y" hours. The E_rC_{50} was calculated by either the moving average method or by non-linear interpolation between the two concentrations which bracket the 50% effect level of a computer program (Stephan:1977, 1982); the program uses percentage effect and the nominal measured test concentration in test samples. The "no observed effect concentrations" (NOEC) was determined using Dunnett's multicomparison test to compare the percentage inhibition in the test group with that for the control cultures (Dunnett:1955, 1964).</p> <p>The measured concentrations of 2-methyl-2-butene ranged between 19 and 27% of their nominal values at the start of the test and between 22 and 29% of nominal after 96 hours. Based on an arithmetic mean, the overall mean measured levels of 2-methyl-2-butene were 0.689, 1.53, 3.61, 7.22 and 21.1 mg/l.</p> <p>Area under the growth curve (measured concentrations): E_bC_{50} (72 h) : 10.5 mg/l (95% confidence limits, 9.55 & 11.7 mg/l) E_bC_{50} (96 h) : 10.1 mg/l (95% confidence limits, 9.21 & 11.1 mg/l) No observed effect concentration (NOEC) : 3.61 mg/l</p> <p>Average specific growth rate (measured concentrations): E_rC_{50} (0-72 h) : 12.0 mg/l (95% confidence limits, 7.22 & 21.1 mg/l) E_rC_{50} (0-96 h) : 13.2 mg/l (95% confidence limits, 12.2 & 14.3 mg/l) No observed effect concentration (NOEC) : 7.22 mg/l</p> <p>After 96 hours of exposure, the majority of the cells at 21.1 mg/l were swollen and/or mis-shapen.</p> <p>After 72 and 96 hours of exposure to 2-methyl-2-butene, the E_bC_{50} values were 10.5 and 13.2 mg/l respectively; the E_rC_{50} values were 12.0 and 13.2 mg/l respectively.</p> <p>The "no observed effect concentration" (NOEC) for area under the growth curve and growth rate respectively, were 3.61 and 7.22 mg/l.</p> <p>(1) Valid without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2003. Algal growth inhibition assay. Project ID CSS 003. Huntingdon Life Sciences Ltd., Cambridgeshire, England</p>
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Alga Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed</p> <p>GLP Year Endpoint Species</p> <p>Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p>	<p>Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content.</p> <p>The Paraffin/Olefin/Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%).</p> <p>Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.</p> <p>OECD Guide-line 201, EC Directive 92/69 C3, US EPA TSCA 797.1050 & 797.1060</p> <p>Yes 2002-2003 Growth rate <i>Pseudokirchneriella subcapitata</i> (formerly <i>Selenastrum capricornutum</i>) (Algae)</p> <p>96 hours No Yes</p> <p>The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Hydrotreated C5s. The test medium was prepared by stirring the test substance in a sealed mixing vessel for approximately 21 hours in the dark. After being allowed to stand for approximately 60 minutes to obtain an equilibrium concentration of the test substance, a portion of the medium was removed from the middle of the vessel and after the inoculation with algal cells, the medium was used to fill replicate test vessels at each concentration. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 22.0 to 23.2°C for 96 hours. Replicate algal cultures, with an initial cell density of 1×10^4/ml, were exposed to Hydrotreated C5s at nominal concentrations of 4.27, 9.39, 20.7, 45.5 and 100 mg/l.</p> <p>The exposure levels were monitored by measuring the concentrations of Hydrotreated C5s in samples of the test media using a GLC method of analysis.</p> <p>Cell densities were measured daily to monitor growth, and the test results are expressed in terms of the area under the growth curve and growth rate.</p> <p>The area under the growth curve and the average specific growth rate are taken to be an index of growth and are calculated mathematically.</p>
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<p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>(1) Valid without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Algal growth inhibition assay. Project ID CSS 023. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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Alga Toxicity

<p><u>Test Substance</u> Remarks</p> <p><u>Method</u> Method/guideline followed</p> <p>GLP Year Endpoint Species</p> <p>Exposure period Limit test Analytical monitoring Study Design</p> <p>Evaluation of data</p>	<p>Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5 rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in the study.</p> <p>OECD Guide-line 201, EC Directive 92/69 C3, US EPA TSCA 797.1050 & 797.1060</p> <p>Yes 2002 Growth rate <i>Pseudokirchneriella subcapitata</i> (formerly <i>Selenastrum capricornutum</i>) (Algae) 96 hours No Yes</p> <p>The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Pyrolysis C5s. The test medium was prepared by stirring the test substance in a sealed mixing vessel for approximately 21 hours in the dark. After being allowed to stand for approximately 90 minutes to obtain an equilibrium concentration of the test substance, a portion of the medium was removed from the middle of the vessel and after the inoculation with algal cells, the medium was used to fill replicate test vessels at each concentration. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 23.1 to 24.4°C for 96 hours. Replicate algal cultures, with an initial cell density of 1×10^4/ml, were exposed to Pyrolysis C5s at nominal concentrations of 4.27, 9.39, 20.7, 45.5 and 100 mg/l.</p> <p>The exposure levels were monitored by measuring the concentrations of Pyrolysis C5s in samples of the test media using an HPLC method of analysis.</p> <p>Cell densities were measured daily to monitor growth, and the test results are expressed in terms of the area under the growth curve and growth rate.</p> <p>The area under the growth curve and the average specific growth rate are taken to be an index of growth and are calculated mathematically.</p> <p>The E_bL_{50}/E_bC_{50} ("x" h) is the median effect concentration for inhibition of growth based on a comparison of areas under the</p>
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	<p>growth curves after "x" hours. The E_bL_{50}/E_bC_{50} was calculated by the moving average method using a computer program (Stephan:1977, 1982); the program uses percentage effect and the nominal loading rates and measured test concentration in test samples.</p> <p>The E_rL_{50}/E_rC_{50} ("x" - "y" h) is the median effect loading rate/concentration for inhibition of growth based on a comparison of growth rates from "x" to "y" hours. The E_rL_{50}/E_rC_{50} was calculated by the moving average method using a computer program (Stephan:1977, 1982); the program uses percentage effect and the nominal loading rate and measured test concentration in test samples.</p> <p>The "no observed effect loading rate/concentration" (NOELR/NOEC) was determined using Dunnett's multicomparison test to compare the percentage inhibition in the test group with that for the control cultures (Dunnett:1955, 1964).</p> <p>The measured concentrations of Pyrolysis C5s ranged between 15 and 32% of their nominal values at the start of the test and between 20 and 38% of nominal after 96 hours. Based on an arithmetic mean, the overall mean measured levels of Pyrolysis C5s were 1.22, 3.26, 6.47, 7.84 and 30.6 mg/l.</p> <p>Area under the growth curve: E_bL_{50} (72 h) : 58.1 mg/l (95% confidence limits, 53.7 & 62.3 mg/l) E_bL_{50} (96 h) : 56.0 mg/l (95% confidence limits, 51.7 & 60.0 mg/l) E_bC_{50} (72 h) : 12.4 mg/l (95% confidence limits, 11.3 & 13.6 mg/l) E_bC_{50} (96 h) : 11.7 mg/l (95% confidence limits, 10.8 & 12.8 mg/l)</p> <p>No observed effect loading rate (NOELR) : 9.39 mg/l No observed effect concentration (NOEC): 3.26 mg/l.</p> <p>Average specific growth rate (measured concentrations): E_rL_{50} (72 h) : 75.6 mg/l (95% confidence limits, 70.4 & 81.9 mg/l) E_rL_{50} (96 h) : 74.4 mg/l (95% confidence limits, 69.6 & 80.0 mg/l) E_rC_{50} (72 h) : 18.9 mg/l (95% confidence limits, 16.7 & 21.7 mg/l) E_rC_{50} (96 h) : 18.4 mg/l (95% confidence limits, 16.4 & 20.8 mg/l)</p> <p>No observed effect loading rate (NOELR) : 45.5 mg/l No observed effect concentration (NOEC) : 7.84 mg/l.</p>
<p><u>Results</u></p> <p>Observations</p>	<p>After 96 hours of exposure, the cells at 100 mg/l (30.6 mg/l, measured) were swollen and/or mis-shapen.</p>

<p><u>Conclusions</u></p> <p><u>Data Quality</u></p> <p><u>Reference</u></p>	<p>After 96 hours of exposure to Pyrolysis C5s, the E_bL_{50}/E_bC_{50} values were 56.0 and 11.7 mg/l respectively; the E_rL_{50}/E_rC_{50} values were 74.4 and 18.4 mg/l respectively.</p> <p>The "no observed effect loading rate/concentration" (NOELR/NOEC) for area under the growth curve respectively were 9.39 and 3.26 mg/l. For growth rate, the NOELR/NOEC respectively were 45.5 and 7.84 mg/l.</p> <p>(1) Valid without restrictions</p> <p>Huntingdon Life Sciences Ltd. 2002. Algal growth inhibition assay. Project ID CSS 013. Huntingdon Life Sciences Ltd., Cambridgeshire, England.</p>
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