

APPENDIX II

ROBUST SUMMARIES OF STUDIES USED TO CHARACTERIZE THE
FUEL OILS CATEGORYPHYSICO-CHEMICAL ROBUST SUMMARIES

Melting Point

Test Substance:	Other TS [CAS # 64741-62-4; 64742-90-1; 68131-05-5; 68409-73-4; 68475-80-9; 68513-69-9; 68514-34-1; 68527-18-4; 68921-67-5; 69013-21-4; 69430-33-7; 8002-05-9; 64742-47-8]
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:	<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. The Gold and Ogle Method is described by Lyman, W.J., 1985, In: <u>Environmental Exposure from Chemicals</u>. Volume 1. Neely, W.B. and Blau, G.E. (eds), Boca Raton, FL, CRC Press, Inc., Chapter 2.</p>

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<p>Results:</p> <p>Units/Value:</p> <ul style="list-style-type: none"> Note: Deviations from protocol or guideline, analytical method. 	<p>Calculated and measured melting point data for representative constituents of the Fuel Oils Category are listed below. The data identify a potential melting point range for substances represented by the 12 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> <p>Commercial substances in this category consist of complex hydrocarbon reaction products with a carbon number distribution that is predominantly in a C8 and higher range. The 1,3-butadiene content is negligible. The five chemicals selected to represent the melting point range of this category are C9-C12 hydrocarbons that can be found in substances identified by the 12 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category chemistry/structure and olefinic process (distillation) knowledge.</p> <table border="1"> <thead> <tr> <th>Substance Constituent</th> <th>Calculated MP (°C)</th> <th>Measured* MP (°C)</th> </tr> </thead> <tbody> <tr> <td>indene</td> <td>24.36</td> <td>na</td> </tr> <tr> <td>dicyclopentadiene</td> <td>-16.78</td> <td>32.0</td> </tr> <tr> <td>naphthalene</td> <td>5.01</td> <td>80.2</td> </tr> <tr> <td>methylnaphthalene</td> <td>22.15</td> <td>34.4</td> </tr> <tr> <td>1,1'-biphenyl</td> <td>25.07</td> <td>69.0</td> </tr> </tbody> </table> <p>* Experimental values from EPIWIN database. na = not available</p> <p>The data represent a potential melting point range for substances represented by the 12 CAS numbers under <u>Test Substance</u>.</p>	Substance Constituent	Calculated MP (°C)	Measured* MP (°C)	indene	24.36	na	dicyclopentadiene	-16.78	32.0	naphthalene	5.01	80.2	methylnaphthalene	22.15	34.4	1,1'-biphenyl	25.07	69.0
Substance Constituent	Calculated MP (°C)	Measured* MP (°C)																	
indene	24.36	na																	
dicyclopentadiene	-16.78	32.0																	
naphthalene	5.01	80.2																	
methylnaphthalene	22.15	34.4																	
1,1'-biphenyl	25.07	69.0																	
<p>Test Substance:</p>	<p>The Fuel Oils Category includes the following CAS numbers:</p> <p>64741-62-4 Clarified oils, petroleum, catalytic cracked 64742-90-1 Residues, petroleum, steam cracked 68131-05-5 Hydrocarbon oils, process blends 68409-73-4 Aromatic hydrocarbons, biphenyl-rich 68475-80-9 Distillates, petroleum, light steam-cracked naphtha</p>																		

	<p>68513-69-9 Residues, petroleum, steam cracked light 68514-34-1 Hydrocarbons, C9-14, ethylene-manufacture-by-product 68527-18-4 Gas oils, petroleum, steam-cracked 68921-67-5 Hydrocarbons, ethylene-manufacture-by-product distillation residues 69013-21-4 Fuel oil, pyrolysis 69430-33-7 Hydrocarbons, C6-30 64742-47-8 Distillates, petroleum, hydrotreated light 8002-05-9 Petroleum</p> <p>The Fuel Oils Category was developed by grouping eight ethylene industry streams made up of hydrocarbons that are C8 and higher with varying amounts of lower boiling materials. The 12 CAS numbers are used to describe the eight process streams arising from the ethylene process and other associated manufacturing processes. The category includes hydrocarbon process streams consisting predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The 1,3-butadiene content of the process streams is negligible.</p> <p>More information on the Fuel Oils 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 Fuel Oils Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.
<p>Conclusion:</p>	<p>The calculated melting points for selected representative constituents that are present in category streams range from -16.78 to 25.07 °C. The measured melting points of these same constituents range from 32.0 to 80.2°C. Although this does not define the actual melting points of the category streams, it offers an indication of a range that might be expected to encompass the melting points of these complex streams with variable compositions. Melting points outside of these ranges may be possible for some category streams.</p>

Reliability:	(2) Reliable with restrictions 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 12 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 Fuel Oils 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.
Reference:	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.)

Boiling Point

Test Substance:	<p>Industry Stream Name: Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation)</p> <table border="0"> <tr> <td><u>CAS Number</u></td> <td><u>CAS Inventory Name</u></td> </tr> <tr> <td>68513-69-9</td> <td>Residues, petroleum, steam-cracked light</td> </tr> <tr> <td>68921-67-5</td> <td>Hydrocarbons, ethylene-manuf.-by-product distn. residues</td> </tr> </table> <p>This stream is separated by distillation from pyrolysis gasoline, as a bottoms product. The composition indicates a carbon number distribution from C9 or C10 to hydrocarbons boiling at 650°F or higher. The reported typical composition includes 20% dicyclopentadiene, 30% codimers of C5 and C6 monomers, 20% naphthalene and substituted naphthalenes.</p>	<u>CAS Number</u>	<u>CAS Inventory Name</u>	68513-69-9	Residues, petroleum, steam-cracked light	68921-67-5	Hydrocarbons, ethylene-manuf.-by-product distn. residues
<u>CAS Number</u>	<u>CAS Inventory Name</u>						
68513-69-9	Residues, petroleum, steam-cracked light						
68921-67-5	Hydrocarbons, ethylene-manuf.-by-product distn. residues						
Method/Guideline:	EEC A2 / OECD 103						
Year (guideline):	1992 / 1995						
Type (test type):	Boiling Point (distillation method)						
GLP:	Yes						
Year (study performed):	2004						
Pressure	Corrected to Standard Atmospheric						
Boiling Point Value:	114 - 248 Deg C						
Test Conditions: <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, test conditions. 	<p>Test substance added to distillation flask and heated at a rate which resulted in initial drops of distillate condensing after 10-15 minutes. On boiling, the heating rate was adjusted in order that the distillation rate was approximately 3 mL/min. Procedure performed in duplicate.</p>						

<p>Results:</p> <p>Units/Value:</p>	<p>Results of duplicate measurements:</p> <p>Run I initial B.P. 115 Deg C final B.P. 249 Deg C</p> <p>Run II initial B.P. 113 Deg C final B.P. 247 Deg C</p> <p>Mean 114 - 248 Deg C</p> <p>A small amount of thick brown residue remained in the flask at the end of the test.</p>
<p>Reliability:</p>	<p>(1) Reliable without restriction</p>
<p>Reference:</p>	<p>Huntingdon Life Sciences, Ltd. 2004, Physicochemical Properties for Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation). Study EXN078/042054.</p>

Boiling Point

Test Substance:	<p>Industry Stream Name: Heavy Pyrolysis Fuel Oil</p> <table border="0"> <thead> <tr> <th><u>CAS Number</u></th> <th><u>CAS Inventory Name</u></th> </tr> </thead> <tbody> <tr> <td>68513-69-9</td> <td>Residues, petroleum, steam-cracked light</td> </tr> <tr> <td>64741-62-4</td> <td>Clarified oils, petroleum, catalytic cracked</td> </tr> <tr> <td>69013-21-4</td> <td>Fuel oil, pyrolysis</td> </tr> <tr> <td>8002-05-9</td> <td>Petroleum</td> </tr> </tbody> </table> <p>In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is quenched by injection of recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil.</p>	<u>CAS Number</u>	<u>CAS Inventory Name</u>	68513-69-9	Residues, petroleum, steam-cracked light	64741-62-4	Clarified oils, petroleum, catalytic cracked	69013-21-4	Fuel oil, pyrolysis	8002-05-9	Petroleum
<u>CAS Number</u>	<u>CAS Inventory Name</u>										
68513-69-9	Residues, petroleum, steam-cracked light										
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8002-05-9	Petroleum										
Method/Guideline:	EEC A2 / OECD 103										
Year (guideline):	1992 / 1995										
Type (test type):	Boiling Point (distillation method)										
GLP:	Yes										
Year (study performed):	2004										
Pressure	Corrected to Standard Atmospheric (test performed at 992 mBar)										
Boiling Point Value:	201 - 340 Deg C										
Test Conditions: <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, test conditions. 	<p>Test substance added to distillation flask and heated at a rate which resulted in initial drops of distillate condensing after 10-15 minutes. On boiling, the heating rate was adjusted in order that the distillation rate was approximately 3 mL/min. The rate decreased as the higher boiling components distilled. Procedure performed in duplicate.</p>										

<p>Results:</p> <p>Units/Value:</p>	<p>Results of duplicate measurements:</p> <p>Run I initial B.P. 201 Deg C final B.P. 339 Deg C</p> <p>Run II initial B.P. 201 Deg C final B.P. 341 Deg C</p> <p>Mean 201 - 340 Deg C</p> <p>Approximately 80% of the test substance distilled over this temperature range, the remainder decomposing at high temperatures. The remaining material formed a hard gray/black mass in the distillation flask indicative of decomposition.</p>
<p>Reliability:</p>	<p>(1) Reliable without restriction</p>
<p>Reference:</p>	<p>Huntingdon Life Sciences, Ltd. 2004, Physicochemical Properties for Heavy Pyrolysis Fuel Oil. Study EXN077/042053.</p>

Boiling Point

Test Substance:	Other TS [CAS # 64741-62-4; 64742-90-1; 68131-05-5; 68409-73-4; 68475-80-9; 68513-69-9; 68514-34-1; 68527-18-4; 68921-67-5; 69013-21-4; 69430-33-7; 8002-05-9; 64742-47-8]
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:	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: Concentration prep., vessel type, replication, test conditions. Note: Deviations from protocol or guideline, analytical method. 	<p>Calculated and measured boiling point data for representative constituents of the Fuel Oils Category are listed below. The data identify a potential boiling point range for substances represented by the 12 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 complex hydrocarbon reaction products with a carbon number distribution that is predominantly in a C8 and higher range. The 1,3-butadiene content is negligible. The five chemicals selected to represent the boiling point range of this category are C9-C12 hydrocarbons that can be found in substances identified by the 12 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category chemistry/structure and</p>

	olefinic process (distillation) knowledge.		
Results: (continued)	Substance	Calculated	Measured*
Units/Value:	<u>Constituent</u>	<u>BP (°C)</u>	<u>BP (°C)</u>
Note: Deviations from protocol or guideline, analytical method.	indene	212.89	na
	dicyclopentadiene	176.78	170.0
	naphthalene	231.64	217.9
	methylnaphthalene	249.60	241.1
	1,1'-biphenyl	272.53	256.1
	* Experimental values from EPIWIN database. na = not available		
	The data represent a potential boiling point range for substances represented by the 12 CAS numbers under <u>Test Substance</u> .		
Test Substance:	<p>The Fuel Oils Category includes the following CAS numbers:</p> <p>64741-62-4 Clarified oils, petroleum, catalytic cracked</p> <p>64742-90-1 Residues, petroleum, steam cracked</p> <p>68131-05-5 Hydrocarbon oils, process blends</p> <p>68409-73-4 Aromatic hydrocarbons, biphenyl-rich</p> <p>68475-80-9 Distillates, petroleum, light steam-cracked naphtha</p> <p>68513-69-9 Residues, petroleum, steam cracked light</p> <p>68514-34-1 Hydrocarbons, C9-14, ethylene-manufacture-by-product</p> <p>68527-18-4 Gas oils, petroleum, steam-cracked</p> <p>68921-67-5 Hydrocarbons, ethylene-manufacture-by-product distillation residues</p> <p>69013-21-4 Fuel oil, pyrolysis</p> <p>69430-33-7 Hydrocarbons, C6-30</p> <p>64742-47-8 Distillates, petroleum, hydrotreated light</p> <p>8002-05-9 Petroleum</p> <p>The Fuel Oils Category was developed by grouping eight ethylene industry streams made up of hydrocarbons that are C8 and higher with varying amounts of lower boiling materials. The 12 CAS numbers are used to describe the eight process streams arising from the ethylene process and other associated manufacturing processes. The category includes hydrocarbon process streams consisting predominantly</p>		

	<p>of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The 1,3-butadiene content of the process streams is negligible.</p> <p>More information on the Fuel Oils 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 Fuel Oils Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.
Conclusion:	<p>The calculated boiling points for selected representative constituents that are present in category streams range from 176.78 to 272.53°C @ 760 mm Hg. The measured boiling points of these same constituents range from 170.0 to 256.1°C @ 760 mm Hg. Although this does not define the actual boiling points of the category streams, it offers an indication of a range that might be expected to encompass the boiling points of these complex streams with variable compositions. Boiling points outside of these ranges may be possible for some category streams.</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 12 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 Fuel Oils 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>
Reference:	<p>EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA. (Boiling point</p>

	values were calculated by the MPBPWIN subroutine and measured data came from the database in the computer program.)
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Partition Coefficient

Test Substance:	<p>Industry Stream Name: Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation)</p> <table border="0"> <tr> <td><u>CAS Number</u></td> <td><u>CAS Inventory Name</u></td> </tr> <tr> <td>68513-69-9</td> <td>Residues, petroleum, steam-cracked light</td> </tr> <tr> <td>68921-67-5</td> <td>Hydrocarbons, ethylene-manuf.-by-product distn. residues</td> </tr> </table> <p>This stream is separated by distillation from pyrolysis gasoline, as a bottoms product. The composition indicates a carbon number distribution from C9 or C10 to hydrocarbons boiling at 650°F or higher. The reported typical composition includes 20% dicyclopentadiene, 30% codimers of C5 and C6 monomers, 20% naphthalene and substituted naphthalenes.</p>	<u>CAS Number</u>	<u>CAS Inventory Name</u>	68513-69-9	Residues, petroleum, steam-cracked light	68921-67-5	Hydrocarbons, ethylene-manuf.-by-product distn. residues
<u>CAS Number</u>	<u>CAS Inventory Name</u>						
68513-69-9	Residues, petroleum, steam-cracked light						
68921-67-5	Hydrocarbons, ethylene-manuf.-by-product distn. residues						
Method/Guideline:	EEC A8 / OECD 117						
Year (guideline):	1992 / 1989						
Type (test type):	N-Octanol/Water Partition Coefficient (HPLC method)						
GLP:	Yes						
Year (study performed):	2004						
Temperature:	25 Deg C						
Log P_{ow} Value:	3.3 - 5.4						
Test Conditions: <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, test conditions. 	<p>Test substance was evaluated at a concentration of 108 mg/L in a mixture of methanol:tetrahydrofuran:water (73:2:25). HPLC analysis was performed on a Hewlett Packard 1050 Liquid Chromatograph with a Luna 5um C8 (15cm x 3mm id) column with a 1 mL/min flow rate (methanol:water (3:1) mobile phase), 10uL injection volume and UV detection at 210 nm. Six reference compounds (with known log P_{ow} values) at concentrations ranging from approximately 42 to 109 mg/L, were analyzed in a combined solution including nitrobenzene (log P_{ow}=1.9), ethylbenzoate (log P_{ow} = 2.6), bromobenzene (log P_{ow}=3.0), benzylbenzoate (log</p>						

	<p>$P_{ow}=4.0$), triphenylamine ($\log P_{ow}=5.7$) and DDT ($\log P_{ow}=6.2$). Additionally, an unretained standard of 4,5-dihydroxynaphthalene-2,7-disulphonic acid, disodium salt was analyzed to determine the system deadtime.</p> <p>Two sets of reference mixture and test substance runs were performed.</p>
<p>Results: Units/Value:</p>	<p>Multiple components detected with $\log P_{ow}$ values between 3.3 and 5.4 (calculated from the mean exponential regression of reference compounds)</p>
<p>Reliability:</p>	<p>(1) Reliable without restriction</p>
<p>Reference:</p>	<p>Huntingdon Life Sciences, Ltd. 2004, Physicochemical Properties for Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation). Study EXN078/042054.</p>

Partition Coefficient

Test Substance:	<p>Industry Stream Name: Heavy Pyrolysis Fuel Oil</p> <table border="0"> <tr> <td><u>CAS Number</u></td> <td><u>CAS Inventory Name</u></td> </tr> <tr> <td>68513-69-9</td> <td>Residues, petroleum, steam-cracked light</td> </tr> <tr> <td>64741-62-4</td> <td>Clarified oils, petroleum, catalytic cracked</td> </tr> <tr> <td>69013-21-4</td> <td>Fuel oil, pyrolysis</td> </tr> <tr> <td>8002-05-9</td> <td>Petroleum</td> </tr> </table> <p>In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is quenched by injection of recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil.</p>	<u>CAS Number</u>	<u>CAS Inventory Name</u>	68513-69-9	Residues, petroleum, steam-cracked light	64741-62-4	Clarified oils, petroleum, catalytic cracked	69013-21-4	Fuel oil, pyrolysis	8002-05-9	Petroleum
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68513-69-9	Residues, petroleum, steam-cracked light										
64741-62-4	Clarified oils, petroleum, catalytic cracked										
69013-21-4	Fuel oil, pyrolysis										
8002-05-9	Petroleum										
Method/Guideline:	EEC A8 / OECD 117										
Year (guideline):	1992 / 1989										
Type (test type):	N-Octanol/Water Partition Coefficient (HPLC method)										
GLP:	Yes										
Year (study performed):	2004										
Temperature:	25 Deg C										
Log P_{ow} Value:	3.4 - 5.0										
Test Conditions: <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, test conditions. 	<p>Test substance was evaluated at a concentration of 118 mg/L in a mixture of methanol:tetrahydrofuran:water (65:10:25). HPLC analysis was performed on a Hewlett Packard 1050 Liquid Chromatograph with a Luna 5um C8 (15cm x 3mm id) column with a 1 mL/min flow rate (methanol:water (3:1) mobile phase), 10uL injection volume and UV detection at 210 nm. Six reference compounds (with known log P_{ow} values) at concentrations ranging from approximately 42 to</p>										

	<p>109 mg/L, were analyzed in a combined solution including nitrobenzene ($\log P_{ow}=1.9$), ethylbenzoate ($\log P_{ow} = 2.6$), bromobenzene ($\log P_{ow}=3.0$), benzylbenzoate ($\log P_{ow}=4.0$), triphenylamine ($\log P_{ow}=5.7$) and DDT ($\log P_{ow}=6.2$). Additionally, an unretained standard of 4,5-dihydroxynaphthalene-2,7-disulphonic acid, disodium salt was analyzed to determine the system deadtime.</p> <p>Two sets of reference mixture and test substance runs were performed.</p>
<p>Results: Units/Value:</p>	<p>Multiple components detected with Log P_{ow} values between 3.4 and 5.0 (calculated from the mean exponential regression of reference compounds).</p>
<p>Reliability:</p>	<p>(1) Reliable without restriction</p>
<p>Reference:</p>	<p>Huntingdon Life Sciences, Ltd. 2004, Physicochemical Properties for Heavy Pyrolysis Fuel Oil. Study EXN077/042053.</p>

Partition Coefficient

Test Substance:	Other TS [CAS # 64741-62-4; 64742-90-1; 68131-05-5; 68409-73-4; 68475-80-9; 68513-69-9; 68514-34-1; 68527-18-4; 68921-67-5; 69013-21-4; 69430-33-7; 8002-05-9; 64742-47-8]
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: <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, 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: Deviations from protocol or guideline, analytical method. 	Calculated and measured log K_{ow} data for representative constituents of the Fuel Oils Category are listed below. The data identify a potential log K_{ow} range for substances represented by the 12 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. Commercial substances in this category consist of complex hydrocarbon reaction products with a carbon number distribution that is predominantly in a C8 and higher range. The 1,3-butadiene content is negligible.

<p>Results: (continued)</p> <p>Units/Value:</p> <p>Note: Deviations from protocol or guideline, analytical method.</p>	<p>The five chemicals selected to represent the log K_{ow} range of this category are C9-C12 hydrocarbons that can be found in substances identified by the 12 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category chemistry/structure and olefinic process (distillation) knowledge.</p> <table border="1" data-bbox="691 499 1351 793"> <thead> <tr> <th>Substance Constituent</th> <th>Calculated log K_{ow} @ 25°C</th> <th>Measured* log K_{ow} @ 25°C</th> </tr> </thead> <tbody> <tr> <td>indene</td> <td>2.88</td> <td>na</td> </tr> <tr> <td>dicyclopentadiene</td> <td>3.16</td> <td>na</td> </tr> <tr> <td>naphthalene</td> <td>3.17</td> <td>3.30</td> </tr> <tr> <td>methylnaphthalene</td> <td>3.72</td> <td>3.86</td> </tr> <tr> <td>1,1'-biphenyl</td> <td>3.76</td> <td>3.98</td> </tr> </tbody> </table> <p>* Experimental values from EPIWIN database. na = not available The data represent a potential log K_{ow} range for substances represented by the 12 CAS numbers under <u>Test Substance</u>.</p>	Substance Constituent	Calculated log K_{ow} @ 25°C	Measured* log K_{ow} @ 25°C	indene	2.88	na	dicyclopentadiene	3.16	na	naphthalene	3.17	3.30	methylnaphthalene	3.72	3.86	1,1'-biphenyl	3.76	3.98
Substance Constituent	Calculated log K_{ow} @ 25°C	Measured* log K_{ow} @ 25°C																	
indene	2.88	na																	
dicyclopentadiene	3.16	na																	
naphthalene	3.17	3.30																	
methylnaphthalene	3.72	3.86																	
1,1'-biphenyl	3.76	3.98																	
<p>Test Substance:</p>	<p>The Fuel Oils Category includes the following CAS numbers:</p> <p>64741-62-4 Clarified oils, petroleum, catalytic cracked 64742-90-1 Residues, petroleum, steam cracked 68131-05-5 Hydrocarbon oils, process blends 68409-73-4 Aromatic hydrocarbons, biphenyl-rich 68475-80-9 Distillates, petroleum, light steam-cracked naphtha 68513-69-9 Residues, petroleum, steam cracked light 68514-34-1 Hydrocarbons, C9-14, ethylene-manufacture-by-product 68527-18-4 Gas oils, petroleum, steam-cracked 68921-67-5 Hydrocarbons, ethylene-manufacture-by-product distillation residues 69013-21-4 Fuel oil, pyrolysis 69430-33-7 Hydrocarbons, C6-30 64742-47-8 Distillates, petroleum, hydrotreated light 8002-05-9 Petroleum</p> <p>The Fuel Oils Category was developed by grouping eight ethylene industry streams made up of hydrocarbons that are C8 and higher with varying amounts of lower boiling materials. The 12 CAS</p>																		

	<p>numbers are used to describe the eight process streams arising from the ethylene process and other associated manufacturing processes. The category includes hydrocarbon process streams consisting predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The 1,3-butadiene content of the process streams is negligible.</p> <p>More information on the Fuel Oils 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 Fuel Oils Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.
Conclusion:	<p>The calculated log K_{ow} for selected representative constituents that are present in category streams range from 2.88 to 3.76 @ 25°C. The measured log K_{ow} of these same constituents range from 3.30 to 3.98 @ 25°C. Although this does not define the actual log K_{ow} of the category streams, it offers an indication of a range that might be expected to encompass the log K_{ow} of these complex streams with variable compositions. Log K_{ow} values outside of these ranges may be possible for some category streams.</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 12 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 Fuel Oils 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:	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.)
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Vapor Pressure

Test Substance:	<p>Industry Stream Name: Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation)</p> <p><u>CAS Number:</u> 68513-69-9 <u>CAS Inventory Name:</u> Residues, petroleum, steam-cracked light 68921-67-5 Hydrocarbons, ethylene-manuf.-by-product distn. Residues</p> <p>This stream is separated by distillation from pyrolysis gasoline, as a bottoms product. The composition indicates a carbon number distribution from C9 or C10 to hydrocarbons boiling at 650°F or higher. The reported typical composition includes 20% dicyclopentadiene, 30% codimers of C5 and C6 monomers, 20% naphthalene and substituted naphthalenes.</p>
Method/Guideline:	EEC A4 / OECD 104
Year (guideline):	1992 / 1995
Type (test type):	Vapor Pressure (static measurement procedure)
GLP:	Yes
Year (study performed):	2004
Temperature:	25 Deg C
Vapor Pressure Value:	400 Pa
Test Conditions: <ul style="list-style-type: none"> • Note: Concentration prep., vessel type, replication, test conditions. 	<p>Test conducted at five temperatures between 303 and 343 Deg K (30 and 70 Deg C). Actual test temperatures were 303.15, 313.15, 323.15, 333.15 and 343.15. Duplicate measurements made at each temperature.</p>

Results: Units/Value:	Mean vapor pressures were as follows: 490 Pa at 303.15 Deg K 750 Pa at 313.15 Deg K 1220 Pa at 323.15 Deg K 1730 Pa at 333.15 Deg K 2320 Pa at 343.15 Deg K 400 Pa at 25 Deg C (calculated from linear regression)
Reliability:	(1) Reliable without restriction
Reference:	Huntingdon Life Sciences, Ltd. 2004, Physicochemical Properties for Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation). Study EXN078/042054.

Vapor Pressure

Test Substance:	<p>Industry Stream Name: Heavy Pyrolysis Fuel Oil</p> <p><u>CAS Number:</u> <u>CAS Inventory Name:</u> 68513-69-9 Residues, petroleum, steam-cracked light 64741-62-4 Clarified oils, petroleum, catalytic cracked 69013-21-4 Fuel oil, pyrolysis 8002-05-9 Petroleum</p> <p>In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is quenched by injection of recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil.</p>
Method/Guideline:	EEC A4 / OECD 104
Year (guideline):	1992 / 1995
Type (test type):	Vapor Pressure (static measurement procedure)
GLP:	Yes
Year (study performed):	2004
Temperature:	25 Deg C
Vapor Pressure Value:	210 Pa
Test Conditions: <ul style="list-style-type: none"> Note: Concentration prep., vessel type, replication, test conditions. 	Test conducted at five temperatures between 303 and 343 Deg K (30 and 70 Deg C). Actual test temperatures were 303.15, 313.15, 323.15, 333.15 and 343.15. Duplicate measurements made at each temperature.

Results: Units/Value:	Mean vapor pressures were as follows: 260 Pa at 303.15 Deg K 510 Pa at 313.15 Deg K 780 Pa at 323.15 Deg K 1240 Pa at 333.15 Deg K 1750 Pa at 343.15 Deg K 210 Pa at 25 Deg C (calculated from linear regression)
Reliability:	(1) Reliable without restriction
Reference:	Huntingdon Life Sciences, Ltd. 2004, Physicochemical Properties for Heavy Pyrolysis Fuel Oil. Study EXN077/042053.

Vapor Pressure

Test Substance:	Other TS [CAS # 64741-62-4; 64742-90-1; 68131-05-5; 68409-73-4; 68475-80-9; 68513-69-9; 68514-34-1; 68527-18-4; 68921-67-5; 69013-21-4; 69430-33-7; 8002-05-9; 64742-47-8]
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 Fuel Oils Category are listed below. The data identify a potential vapor pressure range for substances represented by the 12 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 complex hydrocarbon reaction products with a carbon number distribution that is predominantly in a C8 and higher range. The 1,3-butadiene content is negligible.</p>

	<p>The five chemicals selected to represent the vapor pressure range of this category are C9-C12 hydrocarbons that can be found in substances identified by the 12 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category chemistry/structure and olefinic process (distillation) knowledge.</p>																		
	<table border="1"> <thead> <tr> <th data-bbox="695 537 922 604">Substance Constituent</th> <th data-bbox="930 537 1122 604">Calculated VP (hPa @ 25°C)</th> <th data-bbox="1166 537 1357 604">Measured* VP (hPa @ 25°C)</th> </tr> </thead> <tbody> <tr> <td data-bbox="695 615 781 642">indene</td> <td data-bbox="984 615 1044 642">0.25</td> <td data-bbox="1230 615 1268 642">na</td> </tr> <tr> <td data-bbox="695 653 927 680">dicyclopentadiene</td> <td data-bbox="984 653 1021 680">2.2</td> <td data-bbox="1230 653 1291 680">3.05</td> </tr> <tr> <td data-bbox="695 690 846 718">naphthalene</td> <td data-bbox="984 690 1037 718">0.11</td> <td data-bbox="1230 690 1291 718">0.05</td> </tr> <tr> <td data-bbox="695 728 938 756">methylnaphthalene</td> <td data-bbox="984 728 1089 756">4.60 E⁻²</td> <td data-bbox="1230 728 1336 756">7.33 E⁻²</td> </tr> <tr> <td data-bbox="695 766 862 793">1,1'-biphenyl</td> <td data-bbox="984 766 1089 793">9.99 E⁻³</td> <td data-bbox="1230 766 1336 793">1.19 E⁻²</td> </tr> </tbody> </table> <p>* Experimental values from EPIWIN database. na = not available</p> <p>The data represent a potential vapor pressure range for substances represented by the 12 CAS numbers under <u>Test Substance</u>.</p>	Substance Constituent	Calculated VP (hPa @ 25°C)	Measured* VP (hPa @ 25°C)	indene	0.25	na	dicyclopentadiene	2.2	3.05	naphthalene	0.11	0.05	methylnaphthalene	4.60 E ⁻²	7.33 E ⁻²	1,1'-biphenyl	9.99 E ⁻³	1.19 E ⁻²
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<p>Test Substance:</p>	<p>The Fuel Oils Category includes the following CAS numbers:</p> <p>64741-62-4 Clarified oils, petroleum, catalytic cracked 64742-90-1 Residues, petroleum, steam cracked 68131-05-5 Hydrocarbon oils, process blends 68409-73-4 Aromatic hydrocarbons, biphenyl-rich 68475-80-9 Distillates, petroleum, light steam-cracked naphtha 68513-69-9 Residues, petroleum, steam cracked light 68514-34-1 Hydrocarbons, C9-14, ethylene-manufacture-by-product 68527-18-4 Gas oils, petroleum, steam-cracked 68921-67-5 Hydrocarbons, ethylene-manufacture-by-product distillation residues 69013-21-4 Fuel oil, pyrolysis 69430-33-7 Hydrocarbons, C6-30 64742-47-8 Distillates, petroleum, hydrotreated light 8002-05-9 Petroleum</p> <p>The Fuel Oils Category was developed by grouping eight ethylene industry streams made up of</p>																		

	<p>hydrocarbons that are C8 and higher with varying amounts of lower boiling materials. The 12 CAS numbers are used to describe the eight process streams arising from the ethylene process and other associated manufacturing processes. The category includes hydrocarbon process streams consisting predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The 1,3-butadiene content of the process streams is negligible.</p> <p>More information on the Fuel Oils 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 Fuel Oils Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.
<p>Conclusion:</p>	<p>The calculated vapor pressures for selected representative constituents that are present in category streams range from 9.99 E^{-3} to 2.2 hPa @ 25°C. The measured vapor pressures of these same constituents range from 7.33 E^{-2} to 3.05 hPa @ 25°C. Although this does not define the actual vapor pressures of the category streams, it offers an indication of a range that might be expected to encompass the vapor pressures of these complex streams with variable compositions. Vapor pressure outside of these ranges may be possible for some category streams.</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 12 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 Fuel Oils 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>

Water Solubility

Test Substance:	Other TS [CAS # 64741-62-4; 64742-90-1; 68131-05-5; 68409-73-4; 68475-80-9; 68513-69-9; 68514-34-1; 68527-18-4; 68921-67-5; 69013-21-4; 69430-33-7; 8002-05-9; 64742-47-8]
Method/Guideline:	Calculated values using WSKOWWIN version 1.36, 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:	Water Solubility is calculated by the WSKOWWIN subroutine, which is based on a Kow correlation method described by W. Meylan, P. Howard and R. Boethling in "Improved method for estimating water solubility from octanol/water partition coefficient". <i>Environ. Toxicol. Chem.</i> 15:100-106. 1995.
Results: Units/Value: <ul style="list-style-type: none"> Note: Deviations from protocol or guideline, analytical method. 	Calculated and measured water solubility data for representative constituents of the Fuel Oils Category are listed below. The data identify a potential water solubility range for substances represented by the 12 CAS numbers under <u>Test Substance</u> . Substances in this category do not have a specific water solubility value. Actual water solubility ranges for substances in this category will vary dependent on their loading rate (i.e., weight of test material added to a volume of water).

	<p>Commercial substances in this category consist of complex hydrocarbon reaction products with a carbon number distribution that is predominantly in a C8 and higher range. The 1,3-butadiene content is negligible. The five chemicals selected to represent the water solubility range of this category are C9-C12 hydrocarbons that can be found in substances identified by the 12 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category chemistry/structure and olefinic process (distillation) knowledge.</p> <table border="1"> <thead> <tr> <th>Substance <u>Constituent</u></th> <th>Calculated WS (mg/L @ 25°C)</th> <th>Measured WS* (mg/L @ 25°C)</th> </tr> </thead> <tbody> <tr> <td>Indene</td> <td>372.1</td> <td>na</td> </tr> <tr> <td>dicyclopentadiene</td> <td>51.9</td> <td>na</td> </tr> <tr> <td>naphthalene</td> <td>183.8</td> <td>142.1</td> </tr> <tr> <td>methylnaphthalene</td> <td>54.6</td> <td>41.4</td> </tr> <tr> <td>1,1'-biphenyl</td> <td>44.7</td> <td>29.0</td> </tr> </tbody> </table> <p>* Experimental values from EPIWIN database. na = not available The data represent a potential water solubility range for substances represented by the 12 CAS numbers under <u>Test Substance</u>.</p>	Substance <u>Constituent</u>	Calculated WS (mg/L @ 25°C)	Measured WS* (mg/L @ 25°C)	Indene	372.1	na	dicyclopentadiene	51.9	na	naphthalene	183.8	142.1	methylnaphthalene	54.6	41.4	1,1'-biphenyl	44.7	29.0
Substance <u>Constituent</u>	Calculated WS (mg/L @ 25°C)	Measured WS* (mg/L @ 25°C)																	
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<p>Test Substance:</p>	<p>The Fuel Oils Category includes the following CAS numbers:</p> <ul style="list-style-type: none"> 64741-62-4 Clarified oils, petroleum, catalytic cracked 64742-90-1 Residues, petroleum, steam cracked 68131-05-5 Hydrocarbon oils, process blends 68409-73-4 Aromatic hydrocarbons, biphenyl-rich 68475-80-9 Distillates, petroleum, light steam-cracked naphtha 68513-69-9 Residues, petroleum, steam cracked light 68514-34-1 Hydrocarbons, C9-14, ethylene-manufacture-by-product 68527-18-4 Gas oils, petroleum, steam-cracked 68921-67-5 Hydrocarbons, ethylene-manufacture-by-product distillation residues 69013-21-4 Fuel oil, pyrolysis 69430-33-7 Hydrocarbons, C6-30 64742-47-8 Distillates, petroleum, hydrotreated light 																		

	<p>8002-05-9 Petroleum</p> <p>The Fuel Oils Category was developed by grouping eight ethylene industry streams made up of hydrocarbons that are C8 and higher with varying amounts of lower boiling materials. The 12 CAS numbers are used to describe the eight process streams arising from the ethylene process and other associated manufacturing processes. The category includes hydrocarbon process streams consisting predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The 1,3-butadiene content of the process streams is negligible.</p> <p>More information on the Fuel Oils 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 Fuel Oils Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.
<p>Conclusion:</p>	<p>The calculated water solubility for selected representative constituents that are present in category streams range from 44.7 to 372.1 mg/L @ 25°C. The measured water solubility of these same constituents range from 29.0 to 142.1 mg/L @ 25°C. Although this does not define the actual water solubility of the category streams, it offers an indication of a range that might be expected to encompass the water solubility of these complex streams with variable compositions. Water solubilities outside of these ranges may be possible for some category streams.</p>
<p>Reliability:</p>	<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 12 CAS numbers under <u>Test Substance</u>. This robust summary has a reliability rating of 2 because the data are not for specific</p>

	substances in the Fuel Oils 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.
Reference:	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.)

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

Test Substance:	Other TS [CAS # 64741-62-4; 64742-90-1; 68131-05-5; 68409-73-4; 68475-80-9; 68513-69-9; 68514-34-1; 68527-18-4; 68921-67-5; 69013-21-4; 69430-33-7; 8002-05-9; 64742-47-8]
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
<ul style="list-style-type: none"> • Wave length value (upper/lower) 	
Relative Intensity:	Not applicable
Test Substance Spectrum:	Not applicable
Test Conditions:	Not applicable
<ul style="list-style-type: none"> • Note: Concentration, temperature, test system type, replication, deviations from guideline or protocol 	
Direct Photolysis:	<u>Summary</u>
<ul style="list-style-type: none"> • Results: half-life, % degradation, quantum yield 	<p>In the environment, direct photolysis will not significantly contribute to the degradation of constituent chemicals in the Fuel Oils Category. The Fuel Oils Category includes eight process streams:</p> <ul style="list-style-type: none"> • Heavy Pyrolysis Fuel Oil from the Ethylene Process Unit • Light Pyrolysis Fuel Oil from the Ethylene Process

	<p>Unit</p> <ul style="list-style-type: none"> • Quench Oil from the Ethylene Process Unit Water Quench System • Pyrolysis Fuel Oil from Pyrolysis Gasoline Distillation • Combined Fuel Oil of the Ethylene Process and Pyrolysis Gasoline Units • Combined Fuel Oil from Benzene Hydrodealkylation (HDA) and Pyrolysis Gasoline Units • Hydrotreated Flux Oil • Biphenyl Concentrate <p>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 Fuel Oils Category</u></p> <p>A process stream is a mixture of chemicals that arises from a chemical reaction or separation activity. The category includes hydrocarbon process streams consisting predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The 1,3-butadiene content of the process streams is negligible. Twelve CAS numbers (see <u>Test Substance</u>) identify products derived from these process streams. This grouping of CAS numbers represents hydrocarbon streams with a carbon number distribution that is predominantly C8-C14. That is why this group is considered a category for purposes of the High Production Volume (HPV) Chemical Program, and designated <u>Fuel Oils</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>More information on the Fuel Oils Category can be found</p>
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	<p>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 eight process streams in this category are:</p> <ul style="list-style-type: none">• Heavy Pyrolysis Fuel Oil from the Ethylene Process Unit: In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is further quenched by injection of recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the primary fractionation tower or oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil consisting of C10+ and considerable PAHs.• Light Pyrolysis Fuel Oil from the Ethylene Process Unit: In some cases, a light pyrolysis fuel oil is produced from the oil quench system in an ethylene plant that cracks liquid feedstocks. This stream may be produced as a side draw from the primary fractionation tower. The stream typically has a carbon number distribution of C9 to C14 and the major components are naphthalene (30 to 60%), methyl naphthalenes and other substituted one and two ring aromatics.• Quench Oil from the Ethylene Process Unit Water Quench System: In ethylene plants cracking only gases, the cracking furnace effluent (after heat recovery) may be further quenched with water. This step results in the condensation of a relatively small amount of higher boiling hydrocarbon components that, after stripping to remove lights, may be isolated as the Quench Oils from the Ethylene Process Unit Water Quench System. This stream is predominantly C7 through components boiling at 650°F or higher. The reported composition indicates 0.1% benzene, 5% toluene, 12% C8 aromatics, 5% naphthalene, 10% anthracene and 65% C7-C18 cyclic olefins.
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	<ul style="list-style-type: none">• Pyrolysis Fuel Oil from Pyrolysis Gasoline Distillation: This stream is separated by distillation from pyrolysis gasoline, as a bottoms product. The reported composition indicates a carbon number distribution of from C9 to hydrocarbons boiling at 650°F or higher. The reported typical composition includes 20% dicyclopentadiene, 30% codimers of C5 and C6 monomers, 20% naphthalene and substituted naphthalenes. • Combined Fuel Oil of the Ethylene Process and Pyrolysis Gasoline Units: A single combined fuel oil stream for the ethylene process unit and the pyrolysis gasoline unit is not an uncommon situation for the industry. The carbon number distribution for this stream is generally C10 to compounds with a boiling point of 650°F or higher. At least in some cases, lower carbon number components are reported for the stream, e.g. C5s at approximately 2% and benzene at up to 4%. The major components reported in the stream are typically 25% C9 compounds, 10-47% naphthalene and 4-30% methylnaphthalenes. • Combined Fuel Oil from Benzene Hydrodealkylation (HDA) and Pyrolysis Gasoline Units: Ethylene process operations that include both a pyrolysis gasoline distillation unit and a benzene hydrodealkylation unit may combine the fuel oil streams from these two units resulting in a single isolated product. Fuel oil is produced in the benzene HDA process by the HDA reactors and separated as a distillation bottoms product. The carbon number distribution for this combined fuel stream is C9 through hydrocarbons with a boiling point of 650°F or higher, although relatively low levels of lower carbon number hydrocarbons may be present, e.g. 0.2% benzene. The major components reported in the stream include 11% C9 aromatics to naphthalene, 7.5-12% DCPD, 7-13% naphthalene, 22% methylnaphthalenes, and 25-35% biphenyl. • Hydrotreated Flux Oil: is a hydrotreated fuel oil stream with a carbon number distribution predominantly C10 to hydrocarbons with a boiling point of 650°F or higher. The stream may be
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	<p>produced as distillation bottoms from a pyrolysis gasoline hydrotreater unit. The components in the stream are predominantly aromatics, paraffins and cyclic compounds. This stream differs from the other fuel oils described above in that its diolefin and vinyl aromatic content is very low.</p> <ul style="list-style-type: none">• Biphenyl Concentrate: is a co-product of the benzene hydrodealkylation unit that is isolated by distillation from the HDA reactor effluent. The carbon number distribution for the stream is C7 to C18, with the major component reported to be 65 to 95% biphenyl. <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 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</p>
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	<p>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="649 462 1396 756"> <thead> <tr> <th rowspan="2">Hydrocarbon</th> <th colspan="2">λ below 290 nm</th> <th colspan="2">λ above 290 nm</th> </tr> <tr> <th>λ_{\max}</th> <th>ϵ</th> <th>λ_{\max}</th> <th>ϵ</th> </tr> </thead> <tbody> <tr> <td>Ethylene</td> <td>193</td> <td>10,000</td> <td>-</td> <td>-</td> </tr> <tr> <td>Benzene</td> <td>255</td> <td>215</td> <td>-</td> <td>-</td> </tr> <tr> <td>Biphenyl</td> <td>246</td> <td>20,000</td> <td>-</td> <td>-</td> </tr> <tr> <td>Naphthalene</td> <td>221</td> <td>100,000</td> <td>311</td> <td>250</td> </tr> <tr> <td></td> <td>270</td> <td>5,000</td> <td></td> <td></td> </tr> </tbody> </table> <p>Olefins with one double bond, or two conjugated double bonds, which constitute a significant proportion of the chemicals in the Fuel Oils Category, do not absorb appreciable light energy above 290 nm. The absorption of UV light to cause cis-trans isomerization about the double bond of an olefin occurs only if it is in conjugation with an aromatic ring (2).</p> <p>Products in the Fuel Oils 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>	Hydrocarbon	λ below 290 nm		λ above 290 nm		λ_{\max}	ϵ	λ_{\max}	ϵ	Ethylene	193	10,000	-	-	Benzene	255	215	-	-	Biphenyl	246	20,000	-	-	Naphthalene	221	100,000	311	250		270	5,000		
Hydrocarbon	λ below 290 nm		λ above 290 nm																																
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Indirect Photolysis: <ul style="list-style-type: none"> • Results: type of sensitizer, concentration of sensitizer, rate constant, % degradation, half-life 	Not applicable
Degradation Products: <ul style="list-style-type: none"> • Note: Identification, concentration 	Unknown
Test Substance:	<p>The Fuel Oils Category includes the following CAS numbers:</p> <p>64741-62-4 Clarified oils, petroleum, catalytic cracked 64742-90-1 Residues, petroleum, steam cracked 68131-05-5 Hydrocarbon oils, process blends 68409-73-4 Aromatic hydrocarbons, biphenyl-rich 68475-80-9 Distillates, petroleum, light steam-cracked naphtha 68513-69-9 Residues, petroleum, steam cracked light 68514-34-1 Hydrocarbons, C9-14, ethylene-manufacture-by-product 68527-18-4 Gas oils, petroleum, steam-cracked 68921-67-5 Hydrocarbons, ethylene-manufacture-by-product distillation residues 69013-21-4 Fuel oil, pyrolysis 69430-33-7 Hydrocarbons, C6-30 64742-47-8 Distillates, petroleum, hydrotreated light 8002-05-9 Petroleum</p>
Conclusion:	Not applicable
Reliability:	<p>These data represent a key study for characterizing the potential of substances in the Fuel Oils Category to undergo direct photodegradation.</p>
Reference:	<p>American Chemistry Council, Olefins Panel. 2003. Photodegradation (Direct): Fuel Oils Category. Rosslyn, VA, USA.</p>

Photodegradation (Indirect)

Test Substance:	Other TS [CAS # 64741-62-4; 64742-90-1; 68131-05-5; 68409-73-4; 68475-80-9; 68513-69-9; 68514-34-1; 68527-18-4; 68921-67-5; 69013-21-4; 69430-33-7; 8002-05-9; 64742-47-8]
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 E ⁶ 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 Fuel Oils Category</u></p> <p>The Fuel Oils Category was developed by grouping eight ethylene industry streams made up of hydrocarbons that are C8 and higher with varying amounts of lower boiling materials. The category includes hydrocarbon process streams consisting predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations.</p> <p>Commercial substances in this category consist of complex hydrocarbon reaction products with a carbon number distribution that is predominantly in a C8 and higher range. The 1,3-butadiene content is negligible. This grouping of CAS numbers represents hydrocarbon streams with a carbon number distribution that is predominantly C8-C14. That is why this group is considered a category for purposes of the High Production Volume (HPV) Chemical Program, and designated <u>Fuel Oils</u>.</p> <p>The five chemicals selected to represent the atmospheric oxidation potential of this category are C9-C12 hydrocarbons that can be found in substances identified by the 12 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category chemistry/structure and olefinic process (distillation) knowledge.</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>
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<p>Indirect Photolysis: (cont'd)</p> <p>Results: type of sensitizer, concentration of sensitizer, rate constant, % degradation, half-life</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> <table border="1" data-bbox="686 630 1401 892"> <thead> <tr> <th><u>Chemical</u></th> <th><u>Calculated* half-life (hrs)</u></th> <th><u>OH- Rate Constant (cm³/molecule-sec)</u></th> </tr> </thead> <tbody> <tr> <td>indene</td> <td>53.0</td> <td>2.4 E⁻¹²</td> </tr> <tr> <td>dicyclopentadiene</td> <td>1.1</td> <td>119.2 E⁻¹²</td> </tr> <tr> <td>naphthalene</td> <td>5.9</td> <td>21.6 E⁻¹²</td> </tr> <tr> <td>methylnaphthalene</td> <td>2.3</td> <td>56.5 E⁻¹²</td> </tr> <tr> <td>1,1'-biphenyl</td> <td>18.9</td> <td>6.8 E⁻¹²</td> </tr> </tbody> </table> <p>* Atmospheric half-life values are based on a 12-hr day.</p> <p>More information on the Fuel Oils Category can be found in the American Chemistry Council, Olefins Panel test plan for this category (Olefins Panel, 2001).</p> <p><u>References:</u></p> <ol style="list-style-type: none"> 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. <i>J. Phys. Chem. Ref. Data Monograph No. 1</i>, 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 Fuel Oils Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA. 	<u>Chemical</u>	<u>Calculated* half-life (hrs)</u>	<u>OH- Rate Constant (cm³/molecule-sec)</u>	indene	53.0	2.4 E ⁻¹²	dicyclopentadiene	1.1	119.2 E ⁻¹²	naphthalene	5.9	21.6 E ⁻¹²	methylnaphthalene	2.3	56.5 E ⁻¹²	1,1'-biphenyl	18.9	6.8 E ⁻¹²
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Degradation Products: <ul style="list-style-type: none"> Note: Identification, concentration 	Unknown
Test Substance:	<p>The Fuel Oils Category includes the following CAS numbers:</p> <p>64741-62-4 Clarified oils, petroleum, catalytic cracked 64742-90-1 Residues, petroleum, steam cracked 68131-05-5 Hydrocarbon oils, process blends 68409-73-4 Aromatic hydrocarbons, biphenyl-rich 68475-80-9 Distillates, petroleum, light steam-cracked naphtha 68513-69-9 Residues, petroleum, steam cracked light 68514-34-1 Hydrocarbons, C9-14, ethylene-manufacture-by-product 68527-18-4 Gas oils, petroleum, steam-cracked 68921-67-5 Hydrocarbons, ethylene-manufacture-by-product distillation residues 69013-21-4 Fuel oil, pyrolysis 69430-33-7 Hydrocarbons, C6-30 64742-47-8 Distillates, petroleum, hydrotreated light 8002-05-9 Petroleum</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 1.1 to 53 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 12 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 Fuel Oils 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:	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.
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Hydrolysis (Stability in Water)

Test Substance:	Other TS [CAS # 64741-62-4; 64742-90-1; 68131-05-5; 68409-73-4; 68475-80-9; 68513-69-9; 68514-34-1; 68527-18-4; 68921-67-5; 69013-21-4; 69430-33-7; 8002-05-9; 64742-47-8]
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: <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, volume, replication, deviations from guideline or protocol 	Not applicable
Results: Units/Value: <ul style="list-style-type: none"> Note: Analytical method, observations, half-lives by pH, degradation products 	Not applicable
Test Substance:	The Fuel Oils Category includes the following CAS numbers: 64741-62-4 Clarified oils, petroleum, catalytic cracked 64742-90-1 Residues, petroleum, steam cracked 68131-05-5 Hydrocarbon oils, process blends 68409-73-4 Aromatic hydrocarbons, biphenyl-rich 68475-80-9 Distillates, petroleum, light steam-cracked naphtha 68513-69-9 Residues, petroleum, steam cracked light 68514-34-1 Hydrocarbons, C9-14, ethylene-manufacture-by-product 68527-18-4 Gas oils, petroleum, steam-cracked

	<p>68921-67-5 Hydrocarbons, ethylene-manufacture-by-product distillation residues 69013-21-4 Fuel oil, pyrolysis 69430-33-7 Hydrocarbons, C6-30 64742-47-8 Distillates, petroleum, hydrotreated light 8002-05-9 Petroleum</p> <p>The Fuel Oils Category was developed by grouping eight ethylene industry streams made up of hydrocarbons that are C8 and higher with varying amounts of some lower boiling materials. The 12 CAS numbers are used to describe the eight process streams arising from the ethylene process and other associated manufacturing processes. The category includes hydrocarbon product streams consisting predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The 1,3-butadiene content of the product streams is negligible.</p> <p>More information on the Fuel Oils 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 Fuel Oils 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 constituent chemicals in the Fuel Oils Category. The Fuel Oils Category includes eight process streams:</p> <ul style="list-style-type: none"> • Heavy Pyrolysis Fuel Oil from the Ethylene Process Unit • Light Pyrolysis Fuel Oil from the Ethylene Process Unit • Quench Oil from the Ethylene Process Unit Water Quench System • Pyrolysis Fuel Oil from Pyrolysis Gasoline Distillation • Combined Fuel Oil of the Ethylene Process and Pyrolysis Gasoline Units • Combined Fuel Oil from Benzene Hydrodealkylation (HDA) and Pyrolysis Gasoline

	<p>Units</p> <ul style="list-style-type: none"> • Hydrotreated Flux Oil • Biphenyl Concentrate <p>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 Fuel Oils Category</u></p> <p>A process stream is a mixture of chemicals that arises from a chemical reaction or separation activity. The category includes hydrocarbon process streams consisting predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The 1,3-butadiene content of the process streams is negligible. Twelve CAS numbers (see Test Substance) identify products derived from these process streams. This grouping of CAS numbers represents hydrocarbon streams with a carbon number distribution that is predominantly C8-C14. That is why this group is considered a category for purposes of the High Production Volume (HPV) Chemical Program, and designated Fuel Oils.</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>More information on the Fuel Oils 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 eight process streams in this category are:</p> <ul style="list-style-type: none"> • Heavy Pyrolysis Fuel Oil from the Ethylene Process Unit: In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is further quenched by injection of
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	<p>recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the primary fractionation tower or oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil consisting of C10+ and considerable PAHs.</p> <ul style="list-style-type: none"> • Light Pyrolysis Fuel Oil from the Ethylene Process Unit: In some cases, a light pyrolysis fuel oil is produced from the oil quench system in an ethylene plant that cracks liquid feedstocks. This stream may be produced as a side draw from the primary fractionation tower. The stream typically has a carbon number distribution of C9 to C14 and the major components are naphthalene (30 to 60%), methyl naphthalenes and other substituted one and two ring aromatics. • Quench Oil from the Ethylene Process Unit Water Quench System: In ethylene plants cracking only gases, the cracking furnace effluent (after heat recovery) may be further quenched with water. This step results in the condensation of a relatively small amount of higher boiling hydrocarbon components that, after stripping to remove lights, may be isolated as the Quench Oils from the Ethylene Process Unit Water Quench System. This stream is predominantly C7 through components boiling at 650°F or higher. The reported composition indicates 0.1% benzene, 5% toluene, 12% C8 aromatics, 5% naphthalene, 10% anthracene and 65% C7-C18 cyclic olefins. • Pyrolysis Fuel Oil from Pyrolysis Gasoline Distillation: This stream is separated by distillation from pyrolysis gasoline, as a bottoms product. The reported composition indicates a carbon number distribution of from C9 to hydrocarbons boiling at 650°F or higher. The reported typical composition includes 20% dicyclopentadiene, 30% codimers of C5 and C6 monomers, 20% naphthalene and substituted naphthalenes.
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	<ul style="list-style-type: none"> • Combined Fuel Oil of the Ethylene Process and Pyrolysis Gasoline Units: A single combined fuel oil stream for the ethylene process unit and the pyrolysis gasoline unit is not an uncommon situation for the industry. The carbon number distribution for this stream is generally C10 to compounds with a boiling point of 650°F or higher. At least in some cases, lower carbon number components are reported for the stream, e.g. C5s at approximately 2% and benzene at up to 4%. The major components reported in the stream are typically 25% C9 compounds, 10-47% naphthalene and 4-30% methylnaphthalenes. • Combined Fuel Oil from Benzene Hydrodealkylation (HDA) and Pyrolysis Gasoline Units: Ethylene process operations that include both a pyrolysis gasoline distillation unit and a benzene hydrodealkylation unit may combine the fuel oil streams from these two units resulting in a single isolated product. Fuel oil is produced in the benzene HDA process by the HDA reactors and separated as a distillation bottoms product. The carbon number distribution for this combined fuel stream is C9 through hydrocarbons with a boiling point of 650°F or higher, although relatively low levels of lower carbon number hydrocarbons may be present, e.g. 0.2% benzene. The major components reported in the stream include 11% C9 aromatics to naphthalene, 7.5-12% DCPD, 7-13% naphthalene, 22% methylnaphthalenes, and 25-35% biphenyl. • Hydrotreated Flux Oil: is a hydrotreated fuel oil stream with a carbon number distribution predominantly C10 to hydrocarbons with a boiling point of 650°F or higher. The stream may be produced as distillation bottoms from a pyrolysis gasoline hydrotreater unit. The components in the stream are predominantly aromatics, paraffins and cyclic compounds. This stream differs from the other fuel oils described above in that its diolefin and vinyl aromatic content is very low. • Biphenyl Concentrate: is a co-product of the benzene hydrodealkylation unit that is isolated by distillation from the HDA reactor effluent. The carbon number distribution for the stream is C7 to C18, with the
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	major component reported to be 65 to 95% biphenyl.
	<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. 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.</p> <p>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 Fuel Oils 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 Fuel Oils 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 Fuel Oils Category have a very low potential to hydrolyze, and this degradative process will not contribute to their removal in the environment.</p>

	<p><u>References</u></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 Fuel Oils 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 Fuel Oils Category to undergo hydrolysis.
Reference:	American Chemistry Council, Olefins Panel. 2003. Hydrolysis. Fuel Oils Category. Rosslyn, VA, USA.

Transport / Distribution (Fugacity)

Test Substance:	Other TS [CAS # 64741-62-4; 64742-90-1; 68131-05-5; 68409-73-4; 68475-80-9; 68513-69-9; 68514-34-1; 68527-18-4; 68921-67-5; 69013-21-4; 69430-33-7; 8002-05-9; 64742-47-8]
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 Fuel Oils Category are listed below. The data identify a potential distribution for substances represented by the 12 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 complex hydrocarbon reaction products with a carbon number distribution that is predominantly in a C8 and higher range. The 1,3-butadiene content is negligible. The five chemicals selected to represent the environmental distribution range of this category are C9-C12 hydrocarbons that can be found in substances identified by the 12 CAS numbers. Constituents representing category members were selected on the basis of carbon number as identified by the category chemistry/structure 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="690 1312 1388 1606"> <thead> <tr> <th rowspan="2"><u>Chemical</u></th> <th colspan="4"><u>Calculated*</u> <u>Percent Distribution</u></th> </tr> <tr> <th><u>Air</u></th> <th><u>Water</u></th> <th><u>Soil</u></th> <th><u>Sediment</u></th> </tr> </thead> <tbody> <tr> <td>indene</td> <td>47.61</td> <td>31.05</td> <td>20.86</td> <td>0.46</td> </tr> <tr> <td>dicyclopentadiene</td> <td>98.00</td> <td>0.87</td> <td>1.11</td> <td>0.02</td> </tr> <tr> <td>naphthalene</td> <td>24.47</td> <td>32.28</td> <td>42.28</td> <td>0.94</td> </tr> <tr> <td>methylnaphthalene</td> <td>97.68</td> <td>0.40</td> <td>1.88</td> <td>0.04</td> </tr> <tr> <td>1,1'-biphenyl</td> <td>10.06</td> <td>14.48</td> <td>73.77</td> <td>1.64</td> </tr> </tbody> </table> <p>* Distribution values determined using calculated input data from EPIWIN program</p>	<u>Chemical</u>	<u>Calculated*</u> <u>Percent Distribution</u>				<u>Air</u>	<u>Water</u>	<u>Soil</u>	<u>Sediment</u>	indene	47.61	31.05	20.86	0.46	dicyclopentadiene	98.00	0.87	1.11	0.02	naphthalene	24.47	32.28	42.28	0.94	methylnaphthalene	97.68	0.40	1.88	0.04	1,1'-biphenyl	10.06	14.48	73.77	1.64
<u>Chemical</u>	<u>Calculated*</u> <u>Percent Distribution</u>																																		
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<p>Results: (cont'd)</p> <p>Units/Value:</p> <ul style="list-style-type: none"> Note: Deviations from protocol or guideline, analytical method. 	<p style="text-align: center;">Measured**</p> <p style="text-align: center;"><u>Percent Distribution</u></p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left;"><u>Chemical</u></th> <th style="text-align: center;"><u>Air</u></th> <th style="text-align: center;"><u>Water</u></th> <th style="text-align: center;"><u>Soil</u></th> <th style="text-align: center;"><u>Sediment</u></th> </tr> </thead> <tbody> <tr> <td>indene</td> <td style="text-align: center;">na</td> <td style="text-align: center;">na</td> <td style="text-align: center;">na</td> <td style="text-align: center;">na</td> </tr> <tr> <td>dicyclopentadiene</td> <td style="text-align: center;">98.55</td> <td style="text-align: center;">0.63</td> <td style="text-align: center;">0.80</td> <td style="text-align: center;">0.02</td> </tr> <tr> <td>naphthalene</td> <td style="text-align: center;">42.27</td> <td style="text-align: center;">20.56</td> <td style="text-align: center;">36.33</td> <td style="text-align: center;">0.81</td> </tr> <tr> <td>methylnaphthalene</td> <td style="text-align: center;">98.53</td> <td style="text-align: center;">0.19</td> <td style="text-align: center;">1.25</td> <td style="text-align: center;">0.03</td> </tr> <tr> <td>1,1'-biphenyl</td> <td style="text-align: center;">11.68</td> <td style="text-align: center;">9.15</td> <td style="text-align: center;">77.40</td> <td style="text-align: center;">1.72</td> </tr> </tbody> </table> <p>** Distribution values determined using input data from the EPIWIN program experimental database. na = not available</p>	<u>Chemical</u>	<u>Air</u>	<u>Water</u>	<u>Soil</u>	<u>Sediment</u>	indene	na	na	na	na	dicyclopentadiene	98.55	0.63	0.80	0.02	naphthalene	42.27	20.56	36.33	0.81	methylnaphthalene	98.53	0.19	1.25	0.03	1,1'-biphenyl	11.68	9.15	77.40	1.72
<u>Chemical</u>	<u>Air</u>	<u>Water</u>	<u>Soil</u>	<u>Sediment</u>																											
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<p>Test Substance:</p>	<p>The Fuel Oils Category includes the following CAS numbers:</p> <p>64741-62-4 Clarified oils, petroleum, catalytic cracked</p> <p>64742-90-1 Residues, petroleum, steam cracked</p> <p>68131-05-5 Hydrocarbon oils, process blends</p> <p>68409-73-4 Aromatic hydrocarbons, biphenyl-rich</p> <p>68475-80-9 Distillates, petroleum, light steam-cracked naphtha</p> <p>68513-69-9 Residues, petroleum, steam cracked light</p> <p>68514-34-1 Hydrocarbons, C9-14, ethylene-manufacture-by-product</p> <p>68527-18-4 Gas oils, petroleum, steam-cracked</p> <p>68921-67-5 Hydrocarbons, ethylene-manufacture-by-product distillation residues</p> <p>69013-21-4 Fuel oil, pyrolysis</p> <p>69430-33-7 Hydrocarbons, C6-30</p> <p>64742-47-8 Distillates, petroleum, hydrotreated light</p> <p>8002-05-9 Petroleum</p> <p>The Fuel Oils Category was developed by grouping eight ethylene industry streams made up of hydrocarbons that are C8 and higher with varying amounts of lower boiling materials. The 12 CAS numbers are used to describe the eight process streams arising from the ethylene process and other associated manufacturing processes. The category includes hydrocarbon process streams consisting predominantly of the same higher-boiling hydrocarbons, mostly cyclic olefins and aromatics, but at varying concentrations. The 1,3-butadiene content of the process streams is negligible.</p>																														

	<p>More information on the Fuel Oils 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 Fuel Oils Category. American Chemistry Council, Olefins Panel, HPV Implementation Task Group. VA, USA.
<p>Conclusion:</p>	<p>The partitioning data represent a potential distribution range for substances in the 12 CAS numbers listed under <u>Test Substance</u>. Substances in the Fuel Oils Category are complex hydrocarbon reaction products, and as a result, the potential environmental distribution of these substances is also expected to be complex. Constituent chemicals are calculated to partition either primarily to air or to air, water, and soil with a small percentage to sediment.</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>
<p>Reliability:</p>	<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 12 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 Fuel Oils 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:	Mackay, D.A. DiGuardo, S. Paterson, and C. Cowan. EQC Model Version 1.01. 1997. Available from the Environmental Modeling Centre, Trent University, Canada.
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Biodegradation

Substance:	<p>Industry Stream Name: Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation)</p> <table border="0"> <tr> <td><u>CAS Number</u></td> <td><u>CAS Inventory Name</u></td> </tr> <tr> <td>68513-69-9</td> <td>Residues, petroleum, steam-cracked light</td> </tr> <tr> <td>68921-67-5</td> <td>Hydrocarbon, ethylene-manuf.-by-product distn. residues</td> </tr> </table> <p>This stream is separated by distillation from pyrolysis gasoline, as a bottoms product. The composition indicates a carbon number distribution of from C9 or C10 to hydrocarbons boiling at 650°F or higher. The reported typical composition includes 20% dicyclopentadiene, 30% codimers of C5 and C6 monomers, 20% naphthalenes and substituted naphthalenes.</p>	<u>CAS Number</u>	<u>CAS Inventory Name</u>	68513-69-9	Residues, petroleum, steam-cracked light	68921-67-5	Hydrocarbon, ethylene-manuf.-by-product distn. residues
<u>CAS Number</u>	<u>CAS Inventory Name</u>						
68513-69-9	Residues, petroleum, steam-cracked light						
68921-67-5	Hydrocarbon, ethylene-manuf.-by-product distn. residues						
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						

<p>Test Conditions:</p> <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, replication, test conditions. 	<p>Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 52.67 mg/L and 51.19 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 3.32 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The microbial count of the inoculum was 10^5 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 $22 \pm 1^\circ\text{C}$.</p>
<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>No biodegradation was observed in each of the triplicate test substance systems, therefore the test substance cannot be considered readily biodegradable.</p>

	<u>Sample</u>	% Degradation* <u>(day 28)</u>	Mean % Degradation <u>(day 28)</u>
	Test Substance	7, 3, 12	7
	Na Benzoate	91, 87, 89	89
	* replicate data		
Conclusion:	Not readily biodegradable		
Reliability:	(1)-Reliable without restriction.		
Reference:	ExxonMobil Biomedical Sciences, Inc. 2002. Ready Biodegradability: Manometric Respirometry test. Study # 176994A. Unpublished report.		

Biodegradation

Test Substance:	<p>Industry Stream Name: Heavy Pyrolysis Fuel Oil</p> <table border="0"> <thead> <tr> <th><u>CAS Number</u></th> <th><u>CAS Inventory Name</u></th> </tr> </thead> <tbody> <tr> <td>68513-69-9</td> <td>Residue, petroleum, steam-cracked light</td> </tr> <tr> <td>64741-62-4</td> <td>Clarified oils, petroleum, catalytic cracked</td> </tr> <tr> <td>69013-21-4</td> <td>Fuel oil, pyrolysis</td> </tr> <tr> <td>8002-05-9</td> <td>Petroleum</td> </tr> </tbody> </table> <p>In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is quenched by injection of recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil.</p>	<u>CAS Number</u>	<u>CAS Inventory Name</u>	68513-69-9	Residue, petroleum, steam-cracked light	64741-62-4	Clarified oils, petroleum, catalytic cracked	69013-21-4	Fuel oil, pyrolysis	8002-05-9	Petroleum
<u>CAS Number</u>	<u>CAS Inventory Name</u>										
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69013-21-4	Fuel oil, pyrolysis										
8002-05-9	Petroleum										
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: <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, replication, test conditions. 	<p>Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 50 mg/L and 51 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 3.32 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The</p>										

	<p>microbial count of the inoculum was 10^5 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 administered by direct addition on glass fiber filters 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>Test Conditions (cont'd):</p> <p>Note: Concentration preparation, vessel type, replication, test conditions.</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>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 test substance biodegraded to 29% and cannot be considered readily biodegradable.</p> <table border="1"> <thead> <tr> <th><u>Sample</u></th> <th><u>% Degradation*</u> <u>(day 28)</u></th> <th><u>Mean % Degradation</u> <u>(day 28)</u></th> </tr> </thead> <tbody> <tr> <td>Test Substance</td> <td>33, 31, 22</td> <td>29</td> </tr> <tr> <td>Na Benzoate</td> <td>91, 87, 89</td> <td>89</td> </tr> </tbody> </table> <p>* replicate data</p>	<u>Sample</u>	<u>% Degradation*</u> <u>(day 28)</u>	<u>Mean % Degradation</u> <u>(day 28)</u>	Test Substance	33, 31, 22	29	Na Benzoate	91, 87, 89	89
<u>Sample</u>	<u>% Degradation*</u> <u>(day 28)</u>	<u>Mean % Degradation</u> <u>(day 28)</u>								
Test Substance	33, 31, 22	29								
Na Benzoate	91, 87, 89	89								
Conclusion:	Not readily biodegradable									
Reliability:	(1)-Reliable without restriction.									
Reference:	ExxonMobil Biomedical Sciences, Inc. 2003. Ready Biodegradability: Manometric Respirometry test. Study # 176894A. Unpublished report.									

Biodegradation

<u>Test Substance</u>	Biphenyl Feedstock, CAS #68989-41-3
<u>Method</u>	
Method/guideline followed	OECD guideline 301D; EEC directive 67/548 Annex V part C.6 (84/449/EEC)
Year (guideline)	1984
Type (test type)	Aerobic Aquatic Biodegradation (Closed Bottle Test)
GLP	Yes
Year (study performed)	1993
Inoculum	Domestic activated sewage sludge bacteria from Huntingdon Research Centre sewage treatment plant.
Exposure Period	28 days
<u>Test Conditions</u>	
Note: Concentration prep., vessel type, replication, test conditions.	<p>Biphenyl Feedstock was initially dissolved in chloroform to give a stock solution of 560mg/ml; 10ml aliquots were adsorbed on Whatman GFA glass filter paper and solvent evaporated to dryness. One piece of filter paper was placed in each test bottle prior to filling with inoculated medium. Test bottles included non-inoculated nutrient medium, inoculated nutrient medium, inoculated medium plus blank filter paper, 2mg/l biphenyl feedstock adsorbed on filter paper, 3mg/l sodium benzoate standard, and 2mg/l biphenyl feedstock adsorbed on filter paper +3mg/l sodium benzoate to evaluate inhibitory effects. The nutrient medium consisted of aerated reverse osmosis purified, deionized water, phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride. Activated sewage sludge filtrate was added at a rate of 1 drop of inoculum/liter. Biochemical oxygen demand (BOD) dark glass 280ml culture bottles, fitted with ground glass stoppers, were filled by siphon and firmly stoppered to exclude all air bubbles. Duplicate bottles were prepared in each test and control series to allow single oxygen determination/bottle at 0, 5, 15, and 28 days. The bottles containing biphenyl feedstock on filter paper +sodium benzoate were sampled on day 0 and 28. All bottles were incubated in a water bath at 20±1⁰C; measurements of dissolved oxygen conc. were made with a Yellow Springs BOD meter. Concentrations of biphenyl feedstock or sodium benzoate as mg carbon/L were not provided. Chemical oxygen demand (COD) was determined using a semi-micro sample digestion method. Test substance samples (50µl) dissolved in chloroform were</p>

<p><u>Results</u> Units/Value:</p> <p>Note: Deviations from protocol or guideline, analytical method.</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliabilities</p> <p><u>Reference</u></p>	<p>placed in clean, dry reaction vials and evaporated to dryness. Deionized water (2ml), + sulfuric acid, potassium dichromate, mercuric sulfate, and silver catalyst were added and reaction vial heated at 150⁰C for 2 hrs. COD values were read by spectrophotometer. Control blanks using solvent only and a sodium benzoate standard were also evaluated.</p> <p>Biphenyl feedstock attained 57% biodegradation after 28 days. Sodium benzoate degraded 86% within 28 days. Cultures containing both biphenyl feedstock and sodium benzoate showed an oxygen depletion value 4% higher than anticipated on the basis of separate cultures indicating that test material does not have an inhibitory effect on sewage bacteria.</p> <p>Percentage biodegradation was determined by comparing the oxygen depletion value with the corresponding Theoretical oxygen demand (NO₃)[ThOD(NO₃)]. ThOD(NO₃) was 2.94mgO₂/mg for biphenyl feedstock (supplied by sponsor) and 1.67mg O₂/mg for sodium benzoate. Chemical oxygen demand (calculated) for biphenyl feedstock was slightly lower at 2.38mgO₂/mg; for sodium benzoate COD was 100% ThOD(NO₃).</p> <p>Biphenyl Feedstock is not readily biodegradable. No inhibitory effects on activated sewage bacteria were observed in this assay.</p> <p>1. Reliable without restriction</p> <p>Douglas, M.T. 1993. Biphenyl Feedstock Ready Biodegradability (Closed Bottle Test). CRTC Ref. #97-78. Huntingdon Research Centre Ltd. Cambridgeshire England for Chevron Research and Technology Co., Richmond, CA, USA. Unpublished report.</p>
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HUMAN HEALTH ROBUST SUMMARIES**Acute Toxicity**

<u>Test Substance</u>	Light Pyrolysis Fuel Oil, CAS #68527-18-4. Water-white liquid. Refer to sponsor for purity and stability.
<u>Method</u>	
Method/guideline followed	Standard method (not referenced) with doses based on a previous limit test and two range-finding studies.
Type (test type)	Acute LD ₅₀
GLP	Yes
Year	1984
Species/Strain	Rat, Fischer 344
Sex	Male and female
No. of animals per sex/dose	5
Vehicle	None
Route of administration	Oral
<u>Test Conditions</u>	Rats (74 days old, 113.4-201.2g) were individually housed in screen-bottomed cages in a room with 71.3 ⁰ F temperature, relative humidity of 46.4% and 12 hr light/dark cycle. Chow diet and water were available ad lib. Rats were fasted for 24 hrs prior to dosing at 2.50, 2.75, 3.00 and 3.25g/kg. Rats were observed at 1 and 4 hrs after oral gavage dosing on day 1, and at least once daily thereafter with exception of days 5, 6, 7, 13 and 14 (holidays and week-ends). Observations for mortality and moribundity were performed daily until sacrifice on day 15. Gross necropsies were performed on all rats. Oral LD ₅₀ were calculated by Probit analysis.
<u>Results</u>	
LC ₅₀ with confidence limits	LD ₅₀ combined sexes (95% confidence limits) = 2.89g/kg (2.63-3.28g/kg). Females were somewhat more susceptible than males. No effects seen on body wt. The most frequently observed clinical signs were nasal and ocular discharges, lethargy, and soft stools. Gross necropsies revealed no adverse findings.
Remarks	
<u>Conclusions</u>	LD ₅₀ combined sexes (95% confidence limits) = 2.89g/kg (2.63-3.28g/kg).
<u>Data Quality</u>	
Reliability	1. Reliable without restrictions

<u>References</u>	Rausina, G.A., 1984. Acute oral toxicity study in rats of Light Pyrolysis Fuel Oil. Proj. #2101. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.
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Acute Toxicity

<p><u>Test Substance</u></p>	<p>Light Pyrolysis Fuel Oil, CAS # 68527-18-4. Clear greenish-blue liquid. Refer to sponsor for purity and stability.</p>
<p><u>Method</u> Method/guideline followed Type (test type) GLP Year Species/Strain Sex No. of animals per sex/dose Vehicle Route of administration</p>	<p>Standard method (not referenced) Limit test Yes 1984 Rat, Fischer 344 Male and female 5 None Inhalation (whole body exposure)</p>
<p><u>Test Conditions</u></p>	<p>Rats (11 wks old, 100-172g) were individually housed in stainless steel, screen-bottomed cages in a room maintained at 72.9⁰F, relative humidity of 51% and 12 hr light/dark cycle. Rats received chow diet and water ad lib, except during exposure. One group of 10 rats was exposed to aerosolized test article, generated by a ball jet nebulizer, for 4 hrs. A condensing flask was used to prevent large particles from entering the chamber. Actual chamber concentration was 4.95g/m³ (range 3.89-5.89g/m³) as determined by gas chromatography. Median aerodynamic diameter was 3.2 microns. Rats were observed for clinical signs immediately after exposure, at 1 and 4 hrs post-dose, and daily thereafter over 14 days. Observations for mortality and moribundity were made twice on weekdays and once on weekends. Body wt was determined prior to exposure on day 1, on day 8, and before sacrifice. Gross necropsies were performed on day 15.</p>
<p><u>Results</u> LC₅₀ with confidence limits Remarks</p>	<p>LC₅₀ was not reached at 4.95g/m³. There were no deaths during the study, and no gross pathological changes were seen at necropsy. Immediately after exposure, all rats exhibited hair discoloration, perianal soiling, dry red material around nose/mouth, and rales. Incidences of rales and/or sneezing/wheezing were sporadically seen in both sexes until day 14. Nasal and ocular discharge, red nose, ocular porphyrin, and partial closing of the eyes were also evident during the post-exposure period. There was initial body wt loss in both sexes by day8, with recovery to initial wt or above by day 15.</p>

<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliability</p> <p><u>References</u></p>	<p>No deaths were observed during the study; therefore the LC₅₀ was not reached at 4.95g/m³. Major clinical signs included hair discoloration, nasal discharge, rales, and dry red material around nose/mouth</p> <p>1. Reliable without restrictions</p> <p>Rausina, G.A., 1984. Acute inhalation toxicity study in rats of Light Pyrolysis Fuel Oil. Proj. #2102. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Acute Toxicity

<p><u>Test Substance</u></p> <p><u>Method</u> Method/guideline followed Type (test type) GLP Year Species/Strain Sex No. animals/sex/dose Vehicle Route of administration</p>	<p>EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538⁰C.</p> <p>None specified Acute limit test Yes 1984 Rat/ Sprague-Dawley Male and female 5 None Oral</p>
<p><u>Test Conditions</u></p>	<p>Animals were individually caged and maintained on a 12 hour light/dark cycle, at approximately 70⁰F and 40% relative humidity. Food and water were provided ad lib. Rats were 6-12 weeks of age at study initiation. A single administration of 5 g/kg was given by gavage. Animals were observed for 14 days post-dose. Gross necropsy was conducted at termination.</p>
<p><u>Results</u> LD₅₀ with confidence limits Remarks</p>	<p>LD₅₀ > 5 g/kg Three of the dosed rats died during the post-dosing period (Days 2, 3, and 6). Clinical signs observed in the surviving rats included ataxia, alopecia, abdominal griping, nasal discharge, urinary, fecal, and ano-genital staining, unthrifty coat, hypoactivity, wet and dry rales, and ocular discharge. The surviving rats all gained weight throughout the observation period. There were no significant findings at gross necropsy.</p>
<p><u>Conclusions</u></p>	<p>The test material was judged to be essentially non-toxic.</p>
<p><u>Data Quality</u> Reliability</p>	<p>1. Reliable without restrictions.</p>
<p><u>References</u></p>	<p>McKee, R.H., Biles, R.W., Kapp, R.W. and Hinz, J.P. 1984. The acute toxicity of coal liquefaction-derived</p>

	materials. J. Appl. Toxicol. 4: 198-205.
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Acute Toxicity

<p><u>Test Substance</u></p>	<p>EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538⁰C.</p>
<p><u>Method</u> Method/guideline followed Type (test type) GLP Year Species/Strain Sex No. of animals/sex/dose Vehicle Route of administration</p>	<p>NAS procedure, Draize scoring Acute dermal toxicity Yes 1984 Rabbit/New Zealand White Males and females 6 None Dermal</p>
<p><u>Test Conditions</u></p>	<p>The study was designed to assess potential for systemic toxicity and skin irritation. Animals were individually caged and maintained on a 12 hour light/dark cycle, at approximately 70⁰F and 40% relative humidity. Food and water were provided ad lib. Rabbits were about 12 weeks of age at study initiation. A single administration of 3.16 g/kg was applied to the clipped dorsal surface of the back of 6 rabbits (3 with abraded backs, 3 non-abraded), and covered with a gauze patch under a plastic sleeve. Patches were removed after 24 hours. Observations were made at 24 hrs post-dose and then daily for a total of 14 days; dermal responses were evaluated 30 min. after dosing, and at 2, 3, 7, 10 and 14 days post-dose. A gross necropsy was performed at terminal sacrifice.</p>
<p><u>Results</u> LD₅₀ with confidence limits. Remarks</p>	<p>LD₅₀ > 3.16 g/kg No animals died during the study. Clinical signs observed during the 14-day observation period included emaciation, nasal discharge, soft stool, and alopecia. Dermal observations included severe erythema and moderate edema; positive dermal scores persisted to study termination. Rabbits also exhibited atonia, fissuring, exfoliation, eschar and desquamation. Both males and females exhibited slight weight loss from study initiation to Day 14.</p>

<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliability</p> <p><u>References</u></p>	<p>The test material produced significant dermal irritation and skin injury as evidenced by fissuring, desquamation, and eschar formation.</p> <p>1. Reliable without restrictions</p> <p>McKee, R.H., Biles, R.W., Kapp, R.W. and Hinz, J.P. 1984.. The acute toxicity of coal liquefaction-derived materials. J. Appl. Toxicol. 4: 198-205.</p> <p>National Academy of Sciences, 1977. Principles and procedures for evaluating the toxicity of household substances. Pp. 23-57. Washington, DC, USA.</p> <p>Draize, J.H. <i>et al.</i> 1944. Pharmacol. Exp. Therapeut. 82: 377 –390.</p>
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Acute Toxicity

<p><u>Test Substance</u></p>	<p>Biphenyl Feedstock, CAS #68989-41-3. Dark brown aromatic slurry consisting of crystalline aggregates in a liquid medium. Refer to sponsor for further information.</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>None specified. Comparable to standard study. Acute Yes 1983 Rat, Fischer 344 Males and females 5M, 5F Corn oil Oral</p>
<p><u>Test Conditions</u></p>	<p>Rats were individually housed in metal screen-bottomed cages, provided with chow diet and water ad lib and maintained at 72-78⁰F, with relative humidity of 23-64% and 12 hour light/dark cycle. Doses for the single dose acute LD₅₀ study were established by performing two preliminary range-finding studies and a more definitive limit test at 5 g/kg. This was followed by testing at 2.5, 3.0, 3.5 and 4.0g/kg. Test article was administered in the form of a 30% (w/v) suspension in corn oil. At study initiation, rats were 91 days old for the limit test and 63 days old for the LD₅₀ determination. Rats were fasted for 24 hr before dosing, and were observed daily for 14 days after dosing for clinical signs, moribundity and mortality. Gross necropsies were performed on all rats. LD₅₀ determinations were performed by the method of Litchfield and Wilcoxin.</p>
<p><u>Results</u> LD₅₀ with confidence limits. Remarks</p>	<p>LD₅₀ (95% confidence limits), combined sexes = 3.7g/kg (3.3-4.2) LD₅₀ males = 3.6g/kg (3.08-4.21); LD₅₀ females = >2.5, <4.0g/kg All surviving rats gained weight normally after 7 and 14 days. Deaths/dose are as follows: Males – 2.5g/kg, 0/5; 3.0g/kg, 0/5; 3.5g/kg, 3/5; 4.0g/kg, 4/5; 5g/kg, 5/6. Females – 2.5g/kg, 0/5; 3.0g/kg, 3/5; 3.5 g/kg 0/5; 4.0g/kg, 3/5; 5g/kg, 5/5. Clinical signs included lethargy, excessive lacrimation, dark inguinal stains and diarrhea that persisted</p>

<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliability</p> <p><u>References</u></p>	<p>until death or diminished in the last several days of the study. At gross necropsy, in rats that died during the study, a black oily substance was usually found in the stomach and/or intestines; this finding was not observed in surviving animals necropsied after 14 days.</p> <p>Acute oral LD₅₀ = 3.7g/kg (95% conf. limits 3.3-4.2g/kg). A black oily substance was usually found in the stomach and/or intestines of rats dying during the study.</p> <p>1. Reliable without restriction</p> <p>Rausina, G.A. 1983. Acute oral toxicity study in albino rats, Biphenyl Feedstock. Proj. #2036. Gulf Life Sciences Center, Pittsburgh PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Acute Toxicity

<u>Test Substance</u>	Biphenyl Feedstock, CAS #68989-41-3. Amber slurry with an aromatic odor. Refer to sponsor for further information.
<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	None specified, comparable to standard study Acute limit test Yes 1983 Rabbit, New Zealand White Males and females 5M, 5F None Dermal
<u>Test Conditions</u>	Rabbits (3.1 –3.6 kg) were individually housed in suspended metal screen-bottomed cages, in a room maintained at 72-80 ⁰ F and relative humidity of 34-60%. Neat test material was applied to the shaved abraded skin on backs (4 parallel, lengthwise, epidermal abrasions) at 2g/kg, and the trunk was wrapped with occlusive sheeting. Each rabbit was fitted with an Elizabethan collar to minimize ingestion during the first 24 hrs of exposure, after which time, the collar and wrappings were removed and the test substance wiped off with a dry towel. Observations for mortality and moribundity, clinical signs, and local skin reactions were continued for 14 days. Body wt was recorded prior to testing and at 7 and 14 days. Gross necropsies were performed after sacrifice.
<u>Results</u> LD ₅₀ with confidence limits Remarks	LD ₅₀ was not reached at a single dermal dose of 2g/kg. One treated female rabbit died on day 1 of the study; no clinical signs were observed before the death. Body weights remained stable throughout the study. The other 9 rabbits appeared normal throughout the 14-day observation period. A delayed skin reaction was observed on day 5 when a well-defined skin irritation was noted in all test rabbits. This irritation decreased during the remainder of the study. Desquamation of skin was found after day 8 in 8 of 9 rabbits. Gross necropsies on all rabbits showed no findings attributable to test article administration.

<p><u>Conclusions</u></p>	<p>LD₅₀ was not reached at a single dose of 2g/kg. The test article produced a delayed skin irritation that first appeared at day 5 and resolved by day 12.</p>
<p><u>Data Quality</u> Reliability</p>	<p>1. Reliable without restrictions</p>
<p><u>References</u></p>	<p>Rausina, G.A. 1983. Acute dermal toxicity study in albino rabbits with Biphenyl feedstock. Proj. #2037. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>

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>Biphenyl Feedstock, CAS #68989-41-3. Light brown and waxy as solid; amber color as liquid at 70⁰C. Refer to sponsor for further information.</p> <p>None specified, comparable to standard study Acute limit test Yes 1982 Rat, Fischer 344 Males and females 10 rats (5M, 5F)/group Filtered air Inhalation</p>
<p><u>Test Conditions</u></p>	<p>One group of Fischer 344 rats was exposed to aerosolized test article at a concentration of 3.0g/m³ (actual) for 4 hr on day 1, followed by 13 days of post-exposure observation for mortality, moribundity, and clinical signs of toxicity. Rats were sacrificed on day 14. Food and water were available ad lib. Rats weighed between 152g and 262g at initiation. Body wt was recorded prior to exposure and on days 7 and 14 (prior to sacrifice and necropsy). The test substance was aerosolized with a nebulizer. Melted test article (80⁰C) was poured into the nebulizer maintained at approx. 75⁰C during atmosphere generation. A condensing flask prevented large diameter particles from entering the exposure chamber. Actual chamber concentration was 3.0g/m³ (TWA) that was primarily particulate with mass median aerodynamic diameter of 4.3 microns.</p>
<p><u>Results</u> LC₅₀ with confidence limits. Remarks</p>	<p>LC₅₀ was not reached at 3.0g/m³. No deaths occurred from the single 4 hr exposure to 3.0g/m³, which was the maximum attainable concentration of test article. Immediately after exposure, all rats were covered with crystalline test article; nearly every rat had dry red material around nose and mouth, perianal soiling, clear ocular discharge, porphyrin around the eyes, and discolored fur. Two males and one female showed labored respiration. These symptoms subsided during the observation period, and at sacrifice, only discolored fur was seen. Body wt was</p>

<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliability</p> <p><u>References</u></p>	<p>unchanged. No test article related lesions were detected at necropsy.</p> <p>LC₅₀ was not reached at 3.0g/m³ and no evidence of systemic toxicity was observed.</p> <p>1. Reliable without restrictions</p> <p>Gordan, T. 1982. Acute LC₅₀ inhalation toxicity test in rats with Biphenyl feedstock. Study # 82-086. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Acute Toxicity

<u>Test Substance</u>	Aromatic Pyrolysis Oil, CAS# 64742-90-1. A brown, tarry, highly viscous mixture
<u>Method</u>	
Method/guideline followed	No guidelines specified; comparable to standard study
Type (test type)	Acute limit test
GLP	Yes
Year	1982
Species/Strain	Rat, Fischer 344
Sex	Male and female
No. of animals per sex per dose	5
Vehicle	None
Route of administration	Oral gavage
<u>Test Conditions</u>	Rats (50 days old) were dosed once with undiluted aromatic pyrolysis oil at 5 g/kg. Each rat was observed for clinical signs, mortality and morbidity at 1 and 4 hours after dosing, and daily thereafter for 14 days. Body weight was obtained at initiation and after 7 and 14 days post dosing. At study termination, all rats were sacrificed and gross necropsies performed.
<u>Results</u>	
LD ₅₀ with confidence limits	No mortality occurred during the study. Clinical signs included oily staining around the mouth and yellow staining of the inguinal region. No remarkable findings related to test article administration were observed at necropsy.
Remarks	
<u>Conclusions</u>	Acute oral median lethal dose for aromatic pyrolysis oil was not reached at a dose of 5 g/kg
<u>Data Quality</u>	
Reliability	1. Reliable without restrictions.
<u>References</u>	Rausina, G.A. 1982. Acute oral toxicity test in albino rats using aromatic pyrolysis oil. Proj. #82-114. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.

Acute Toxicity

<u>Test Substance</u>	Aromatic Pyrolysis Oil, CAS #64742-90-1. A brown, tarry, highly viscous mixture.
<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	No guidelines specified, comparable to standard study. Acute limit test Yes 1982 Rat, Fischer 344 Male and female 5 Filtered air Whole body inhalation
<u>Test Conditions</u>	One group of 10 individually housed rats (5M, 5F; 16-17wks old) was exposed to the aerosolized test substance for 4 hours in stainless steel dynamic exposure chambers, followed by 14 days of post exposure observation for clinical signs, morbidity and death. Body weight was taken before exposure, and 7 and 14 days post exposure. Nominal chamber concentration was 26.8 g/m ³ (uncorrected for large particle condensation) and actual concentration was 3.7 g/m ³ as determined by fluorescence.
<u>Results</u> LC ₅₀ with confidence limits. Remarks	LC50 was not reached at the dose of 3.7 g/m ³ . During exposure, males and females showed excessive tearing and nasal discharge that were absent by day 2 post-exposure. At day 2 and persisting for several days were observations of porphyria around the eyes and ocular discharge. At day 14, the only clinical findings were hair loss and hair discoloration. There were no changes in body weight gain, no deaths, and no abnormal findings at necropsy.
<u>Conclusions</u>	LC50 was not reached at 3.7 g/m ³ .
<u>Data Quality</u> Reliability	1. Reliable without restrictions
<u>References</u>	Gordon, T. 1982. LC50 Aromatic Pyrolysis Oil inhalation study in rats. Proj. #82-082. Gulf Life Sciences Center,

	Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.
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Acute Toxicity

<p><u>Test Substance</u></p>	<p>Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+</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>None specified, comparable to standard studies Acute Not specified 1977 Rat, Wistar Males and females 5 None Oral gavage</p>
<p><u>Test Conditions</u></p>	<p>Male and five female Wistar rats (200-300g) were fasted for 18 hr prior to test article administration by the oral route. Test material was given by intubation at 6.67, 10.0, 15.0 and 22.5g/kg. Following intubation, rats were returned to their holding cages. Food and water were available ad lib. Rats were observed for signs of toxicity and pharmacological effects for 14 days following treatment. Data for the 15.0g/kg dose was taken from a prior project (#MB77-1855).</p>
<p><u>Results</u> LD₅₀ with confidence limits. Remarks</p>	<p>LD₅₀ combined sexes = 14.5 (11.5-18.3)g/kg. Clinical signs were produced at all doses, but the time after dosing when symptoms were seen, was not reported. Effects at the 4 dose doses were: 6.67g/kg, diarrhea, piloerection, lethargy, ptosis; 10.0g/kg, lethargy, ataxia, tremors, coma, flaccid tone, piloerection; 15.0g/kg, lethargy, coma; and 22.5g/kg, coma, death. Rats died at 15.0g/kg (2M, 4F) and 22.5g/kg (5M, 5F).</p>
<p><u>Conclusions</u></p>	<p>LD₅₀ combined sexes = 14.5 (11.5-18.3)g/kg. Toxicological effects were observed at all doses, but death did not occur at doses below 15.0g/kg.</p>
<p><u>Data Quality</u></p>	

<p>Reliability</p> <p><u>References</u></p>	<p>2. Reliable with restrictions. Observation times, when symptoms appeared, were not reported.</p> <p>Moreno, O.M., 1977. Report on oral LD₅₀ in rats. Project #MB77-2027, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp, Paulsboro, NJ, USA.</p> <p>Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ, USA.</p> <p>Moreno, O.M., 1977. Report on a single dose oral toxicity study in rats. Project #MB77-1855, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp, Paulsboro, NJ, USA.</p>
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Acute Toxicity

<p><u>Test Substance</u></p>	<p>Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+</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>None specified, comparable to standard studies Limit test Not specified 1977 Rat, Wistar Males and females 5 None Oral gavage</p>
<p><u>Test Conditions</u></p>	<p>Five male and five female Wistar rats (200-300g) were fasted for 18 hr prior to test article administration (neat) by the oral route. Test material was given by intubation at 15g/kg. Following intubation, rats were returned to their holding cages and observed for signs of toxicity and pharmacological effects for 14 days.</p>
<p><u>Results</u> LD₅₀ with confidence limits Remarks</p>	<p>LD₅₀ combined sexes ~ 15g/kg (Estimated by reviewer) Two of 5 males died on days 1 and 5 (one each day) and 4 of 5 females died on day 2 (2 rats), day 3 (1 rat); day 5 (1 rat). Lethargy and coma were observed shortly after dosing. Higher mortality was seen in females</p>
<p><u>Conclusions</u></p>	<p>The combined-sex LD₅₀ was approximately that of the 15mg/kg limit dose administered, with higher mortality seen in females. This dose is very high and approximates the maximum dose that could be placed in the rat stomach without causing aspiration. (Reviewer's comment)</p>
<p><u>Data Quality</u> Reliability</p>	<p>2. Reliable with restrictions. Very little data was provided in the report related to housing conditions and time course of clinical symptoms. GLP adherence not reported.</p>

<p><u>References</u></p>	<p>Moreno, O.M., 1977. Report on a single dose oral toxicity study in rats. Project #MB77-1855, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp, Paulsboro, NJ, USA.</p> <p>Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ, USA.</p>
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Acute Toxicity

<u>Test Substance</u>	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+.
<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	None specified, comparable to standard studies Acute Yes 1980 Rat, Sprague Dawley Males and females 5 Air Whole body Inhalation
<u>Test Conditions</u>	Five groups of Sprague Dawley rats were exposed to an aerosol of test article for a single 4 hr period at doses of 3.6±0.39, 5.2±0.08, 5.7±0.17, 8.9±0.88 and 9.3±0.79 g/m ³ ±SD. The aerosol had an equivalent aerodynamic diameter of 2.7µm±1.9 geometric SD. Rats were observed during exposure and daily thereafter for clinical signs over 14 days. Body wt was determined at initiation and at 7 and 14 days post-exposure. All rats were necropsied for gross abnormalities.
<u>Results</u> LC ₅₀ with confidence limits Remarks	LC ₅₀ combined sexes ~ 6.0g/m ³ During the exposure, dyspnea, salivation, nasal discharge, and prostration were observed. Dyspnea was observed in practically all rats at all concentrations. Salivation occurred in 8 or more animals exposed to 8.9g/m ³ or greater and to a lesser degree in animals exposed to 5.7g/m ³ . Nasal discharge was observed in all groups to varying degrees. Prostration occurred in 6 or more rats given 5.7g/m ³ or greater and to a lesser degree in animals exposed to 5.2g/m ³ or less; prostration appeared to be concentration related. Males in groups exposed to 3.6 - 8.0g/m ³ weighed slightly more than pre-exposure wt at 7 days post-exposure; by 14 days post-exposure, body weight of these males was approx. normal. Females in groups

<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliability</p> <p><u>References</u></p>	<p>given 3.6 and 5.2g/m³ gained body wt at approx. a normal rate through 14 days post-exposure period. In the 5.7g/m³ group females were below pre-exposure levels at 7 days post-exposure; by 14 days post-exposure, these females were at approx. normal body wt. The females in the 8.9g/m³ group and both males and females in the 9.3g/m³ group did not provide sufficient numbers of surviving animals to evaluate effects on body weight gain. At necropsy, a number of macroscopic abnormalities were observed but only those involving lung, liver and stomach were considered remarkable (specifics not provided).</p> <p>LC₅₀ combined sexes ~ 6.0g/m³</p> <p>2. Reliable with restrictions. The short report provided for review did not describe housing conditions, method of aerosol generation, and specifics on gross necropsy observations.</p> <p>LC₅₀ Acute inhalation toxicity evaluation in rats. 1980. IRDC, Mattawan, MI, for Mobil Chemical Co., Beaumont, TX, USA.</p>
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Acute Toxicity

<u>Test Substance</u>	140 ⁰ F vapor of Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Vapors were 30-35% alkanes/alkenes, 60-65% benzene derivatives, and 2-3% naphthalenes. Benzene, toluene, cyclopentadiene and styrene were 53% of the vapor.
<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	None specified, comparable to standard studies Acute Yes 1983 Rat, Sprague Dawley Males and females 10 Air Whole body Inhalation
<u>Test Conditions</u>	Male and female rats (13wk old; males 349-368g, females 213-215g) were housed 5/sex/cage, in polypropylene cages with removable mesh tops and floors. Chow diet and water were available ad lib. Holding room temperature was 22±0.8 ⁰ C, relative humidity was 63±3.0%, and a 12hr light-dark cycle was maintained. Vapors were continuously generated from a supply of neat oil at 140 ⁰ F in a double-surface glass condenser. Nitrogen gas carrier was passed through the condenser, diluted with air, and metered into the exposure chambers. Chamber vapor concentrations were monitored by an infrared gas analyzer, and samples were collected every 30 min for analysis by gas chromatography/flame ionization detection. Rats received a single 4hr exposure to vapor (g/m ³ ±% variation) at analyzed concentrations of 0.59±21, 3.3±21, or 6.6±21. During exposure, rats were held in stainless steel mesh cages placed on supports in the inhalation chamber. After exposure, rats were returned to holding cages; 5 rats/sex/group were retained for a 24hr observation period, remaining 5 rats/sex/group were retained for a 14 day observation period. Rats were observed for clinical signs at 15 min intervals during exposure, 1 and 2 hrs after exposure, and once daily thereafter until sacrifice. Body wt. was recorded at initiation (day 0), on day 1, and at sacrifice; rats selected for 2wk observation were also weighed on days 2, 4, 7, and

<p><u>Results</u> LC₅₀ with confidence limits Remarks</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliability</p> <p><u>References</u></p>	<p>14. Necropsies were performed on all rats. For the 24hr post-exposure sacrifice, liver, head, lungs and kidneys from all rats were weighed and processed for microscopic examination. For the 2 wk post-exposure sacrifice, lungs were weighed and processed for microscopic examination. Tissues collected from only the high-dose and control rats were examined. Final and initial body wt and organ wt were analyzed by covariance or analysis of variance, as appropriate with application of Bartlett's test.</p> <p>LC₅₀ was not reached at 6.6g/m³ During exposure, visible rats showed partial eye closing and prone body posture in the 3.3 and 6.6g/m³ groups. Slight lacrimation and abnormal breathing was noted in most rats at the high dose. During the 14-day observation period, appearance and behavior were considered normal. There were no statistically significant changes in body wt. At the 24hr post-exposure necropsy, no gross abnormalities were observed. There were no biologically significant changes in organ wt, with the exception of higher kidney wt in the 6.6g/m³ males (p<0.05). There were no significant differences in lung wt at the 24hr or 14-day sacrifices. There were prominent intracytoplasmic eosinophilic inclusions in the renal cortical tubules of males sacrificed at 24hr. No treatment-related effects were seen in the respiratory tract at 24hr or 14-day sacrificed rats. No other significant test article-related microscopic effects were found.</p> <p>LC₅₀ was not reached at 6.6g/m³ No deaths occurred during the study. Clinical signs were indicative of the presence of an irritant. The only biologically significant finding was microscopic eosinophilic inclusions in the renal cortical tubules of high dose males sacrificed 24hr after exposure.</p> <p>1. Reliable without restrictions.</p> <p>Rose, P.H., Jackson, G.C., Clark, G.C., and Gopinath, C. 1983. Acute inhalation toxicity in rats, 4-hour exposure. Report #MOB 9/83503. Huntingdon Research Center plc, Huntingdon, England, for Mobil Oil Corp., Princeton, NJ, USA.</p>
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	Roy, T.A. 1983. Analysis of rerun tower bottoms. Study #1271-81B,C. Mobil Oil Corp, Princeton, NJ, USA.
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Acute Toxicity

<p><u>Test Substance</u></p>	<p>Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+</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>None specified, comparable to standard studies Acute Not specified 1977 Rabbits, New Zealand White Not specified 10 rabbits/dose None Dermal</p>
<p><u>Test Conditions</u></p>	<p>Ten New Zealand White rabbits were clipped free of abdominal hair and 6-10 epidermal abrasions were made longitudinally every 2-3cm over the exposed area. The abrasions were sufficiently deep to penetrate the stratum corneum but not deep enough to produce bleeding. A single 5.0g/kg dose was applied to the exposed area, which was then covered with gauze, and the trunk was wrapped with impervious material for 24 hrs. At 24 hr, the rabbits were cleansed and dermal reactions were evaluated by the Draize technique. Rabbits were then observed for 14 days.</p>
<p><u>Results</u> LC₅₀ with confidence limits Remarks</p>	<p>One rabbit died on day 8 of the study (dosing was on day 0). All erythema and edema scores were between 1 and 2.</p>
<p><u>Conclusions</u></p>	<p>The test material was considered nontoxic by the dermal route.</p>
<p><u>Data Quality</u> Reliability</p>	<p>1. Reliable without restrictions. .</p>
<p><u>References</u></p>	<p>Moreno, O.M., 1977. Acute dermal toxicity in rabbits. Project #MB77-1855, MB Research Laboratories, Inc., Spinnerstown, PA for Mobil Oil Corp., Paulsboro, NJ, USA.</p>

	<p>Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ, USA.</p>
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Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> <i>Test substance</i></p>	<p>Light Pyrolysis Fuel Oil, Gulf. CAS #68527-18-4. Water-white liquid. Composition analysis, purity and stability referred to sponsor.</p>
<p><u>Method</u> Method/guideline followed Type System of testing GLP Year Species/Strain</p>	<p>Standard method based on Hsie <i>et al.</i> (1981), O'Neill & Hsie (1979) In vitro mammalian cell forward mutation Chinese hamster ovary (CHO) cell culture Yes 1985 CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGPRT+/-) from Oak Ridge National Laboratory, TN.</p>
<p>Metabolic activation Species and cell type</p>	<p>Yes Rat liver (S9) fraction purchased from Litron Labs, Rochester, NY</p>
<p>Quantity</p>	<p>1.0mg S9 fraction/ml treatment medium in range finding trial; 0.5mg S9 fraction/ml medium in mutagenicity test</p>
<p>Induced or not induced Concentrations tested</p>	<p>Aroclor 1254 induced (treatment not specified) Range finding (Cytotoxicity): 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 5000µg/ml ± S9; Mutagenicity test #1: (8, 16 cytotoxicity only), 25, 32, 50, 64, 128µg/ml ±S9; repeat +S9: 50, 64µg/ml, all diluted in 50% Pluronic[®] polyol F127 (prepared in absolute ethanol, mol. wt. 12,500).</p>
<p>Statistical Methods</p>	<p>Frequency of mutant colonies per million clonable cells, corrected for absolute survival by viability plates, was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979) using the MUTANT computer program (Snee <i>et al.</i>, 1981). Criteria for positive results were significant (p<0.05) increase in mutant colonies (HGPRT+/- → HGPRT-/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal.</p>
<p><u>Test Conditions</u></p>	<p>Sufficient Light Pyrolysis Fuel Oil (LPFO) was weighed separately for each dose level into 1ml volumetric flasks and stored overnight. The following day, 0.18ml of 50% F127 was added to each flask along with sufficient medium (Ham's F-12 without hypoxanthine) to achieve final 1ml</p>

	<p>volume for testing. All dosing preparations were vortexed just after addition of medium and just prior to use. All cultures were incubated at 37°C in 5% CO₂ enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for –S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For range finding (cytotoxicity), each dose group was composed of 2 flasks, one –S9, one+S9, negative controls ± S9, seeded with 5x10⁵ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. An S9 concentration test using both viability and mutagenicity plates was performed at doses of 16 or 50µg/ml LPFO and S9 concentrations of 0.25, 0.5, 1.0mg/ml to select optimal S9 concentration for the definitive mutagenicity test. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10⁶ cells were exposed to LPFO for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10⁵-10⁶ cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and 2x10⁵ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10⁻⁵M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17 when they were fixed and stained. For mutagenicity, a ratio of total colony counts in mutagenicity plates over absolute survival in viability plates was calculated for each treatment group. Frequency of mutant colonies/million clonable cells was calculated and statistical comparisons with negative control data were made. A repeat mutagenicity test with metabolic activation at two doses was also performed</p>
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	<p>Fuel Oil. Proj. #2105. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA.</p> <p>Hsie, A.W. <i>et al.</i> 1981. <i>Mut. Res.</i> 86: 193-214.</p> <p>O'Neill, J.P. and Hsie, A.W. 1979. <i>Banbury Report 2</i>: 55-63.</p> <p>Irr, J.D. and Snee, R.D. 1979. <i>Banbury Report 2</i>: 263-275. Snee, R.D., Smith, R.L., and Irr, J.D. 1981. <i>MUTANT</i>. A computer program for the evaluation of short-term mutation test results. E.I. Dupont de Nemours Co. Unpublished report.</p>
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Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> <i>Test substance</i></p>	<p>Light Pyrolysis Fuel Oil, Gulf. CAS #68527-18-4. water white liquid. Composition analysis, purity and stability referred to sponsor.</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 Exposure period Statistical Methods</p>	<p>Standard method based on Williams <i>et al.</i> (1977,1982) In vitro mammalian cell DNA repair assay Unscheduled DNA Synthesis (UDS) in primary hepatocyte cultures. Yes 1984 Fischer 344 male rat (8 wks old) No NA NA NA 8, 16, 32, 64, 128, 256, 512, 1024 µg/ml, diluted in 50% Pluronic[®] polyol F127 (prepared in absolute ethanol, mol. wt 12,000, 70% hydrophilic) 18 hours None specified. Criteria for positive response are incorporation of radioactive precursor (³H-thymidine) in cells that are not normally synthesizing DNA, indicating repair of damage. A positive response is defined as a mean net nuclear grain count at any treatment level that exceeds concurrent negative control by at least 6 grains/nucleus; negative control value must not exceed 5 grains. If this criterion is not met, a positive response can be identified if there is a significant difference (p<0.01) in % cells in repair at any dose level and negative control value. This indicator defines whether a small fraction of cells is undergoing repair (Casciano & Gaylor, 1983). A positive response need not be dose related.</p>
<p><u>Test Conditions</u></p>	<p>Sufficient Light Pyrolysis Fuel Oil (LPFO) was weighed separately for each dose level, 0.46ml of 50% F127 added per ml of final volume and sufficient medium (Williams Medium E with 10% fetal bovine serum and insulin) added to achieve final volume. Test preparations were mixed just prior to addition at 30µl to each 3ml culture. The conc. of ³H-thymidine (½ life 12.5 yrs.) used in these assays was 1mCi/ml. All cultures were incubated at 37°C in 5% CO₂ enriched humidified atmosphere. No range finding assay</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>was performed. In the UDS assay, 2×10^5 cells/ml were seeded into coverslip cultures, exposed to ^3H-thymidine and test substance for 18 hours (3 cultures/dose level, 8 dose levels), untreated controls, vehicle F127 control and positive control, 2-acetyl aminofluorene ($0.01 \mu\text{g/ml}$). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at $2-8^\circ\text{C}$. Technical error required the emulsion step be repeated on day 15; first emulsion was peeled off and slides were dipped in fresh emulsion. Autoradiographs were developed, stained and coverslipped on day 22. Numbers of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted and this number was divided by a conversion factor of 2 to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count \div 50) and mean net nuclear grain count (avg. net nuclear grain count/slide \div 3) were calculated. In addition, % cells in repair was determined for each dose level.</p> <p>LPFO induced significant toxicity in primary hepatocytes following 18 hours exposure, leaving too few cells for UDS analysis at doses $\geq 64 \mu\text{g/ml}$. LPFO caused dose related UDS at all non-toxic levels. Percentage of cells in repair increased from the vehicle control value of 2.7% to 43.3% at $8 \mu\text{g/ml}$ to 96.0% at $32 \mu\text{g/ml}$. Statistics were not applied because LPFO induced avg. net nuclear counts >6 grains above neg. controls at all non-toxic levels. Positive and negative controls gave expected responses.</p> <p>Light Pyrolysis Fuel Oil induced dose related unscheduled DNA synthesis in primary cultures of rat hepatocytes beginning at the lowest dose tested. Light Pyrolysis Fuel Oil causes DNA damage and repair under conditions of this assay.</p> <p>2. Reliable with restrictions. Limited specific data supplied. Statistical criteria described but statistical method not cited or employed</p> <p>Brecher, S. 1984. Hepatocyte primary culture/DNA repair test of light pyrolysis fuel oil. Proj. #2107. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA.</p>
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	<p>Williams, G.M. 1977. <i>Cancer Res.</i> 37: 1845-1851.</p> <p>Williams <i>et al.</i> 1977. <i>In Vitro</i> 13: 809-817.</p> <p>Williams <i>et al.</i> 1982. <i>Mut. Res.</i> 97:359-370.</p> <p>Casciano, D.A. and Gaylor, D.W. 1983. <i>Mut. Res.</i> 122:81-86.</p>
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Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> <i>Test substance</i></p>	<p>Light Pyrolysis Fuel Oil, Gulf. CAS #68527-18-4. Water-white liquid. Composition analysis, purity and stability referred to sponsor.</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 Exposure period Statistical Methods</p>	<p>Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973) In vitro cell transformation Mouse embryo cells Yes 1984 BALB/3T3-A31-1-1 from T. Kakunaga, National Cancer Inst., 1982 No NA NA NA Cytotoxicity: 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 5000µg/ml; Transformation: 20, 60, 90, 110µg/ml, all diluted in 50% Pluronic[®] polyol F127 (prepared in absolute ethanol, mol. wt. 12,000, 70% hydrophilic). 2 days None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose.</p>
<p><u>Test Conditions</u></p>	<p>Sufficient Light Pyrolysis Fuel Oil (LPFO) was weighed separately for each dose level, 0.46ml of 50% F127 added per ml of final volume and medium (Eagles MEM with 10% heat-inactivated fetal calf serum) added as required to achieve final volume for testing. Test preparations were mixed just prior to addition to cultures at 50µl to each 5 ml culture. All cultures were incubated at 37⁰C in 5% CO₂ enriched humidified atmosphere. For cytotoxicity, 2 cultures/dose group, 2 cultures for vehicle F127 or medium negative control were seeded with 1x10⁴ cells/culture in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 10% survival. For</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>transformation, 15 cultures (1x10⁴ cells/culture/dose group) and two colony formation cultures (100 cells/culture/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For transformation cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1µg/ml). Colony formation cultures were fixed, stained, and counted visually on day 9 to determine cloning efficiency (avg. number colonies/plate ÷ 100 cells seeded). Transformation cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group.</p> <p>LPFO induced toxicity in BALB/3T3 cells after two days exposure beginning at 32µg/ml (74.8% relative survival); viability dropped sharply at 128µg/ml (6% relative survival) and was 100% toxic at higher concentrations. LPFO did not induce transformed foci in excess of negative control cultures at any dose level. Toxicity was evident at 60µg/ml (10% relative cloning efficiency). Positive and negative controls gave expected responses.</p> <p>Light Pyrolysis Fuel Oil did not induce transformation in BALB/3T3 cells at any dose level under conditions of this assay.</p> <p>1. Reliable without restriction.</p> <p>Brecher, S. 1984. Cell transformation test of Light Pyrolysis Fuel Oil. Proj. #2108. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX, USA.</p> <p>Cortesi, E. <i>et al.</i> 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110.</p> <p>Dunkel, V.A. <i>et al.</i> 1981. J. Nat'l Cancer Inst. 67: 1303-1315.</p> <p>Reznikoff, C.A. <i>et al.</i> 1973. Cancer Res. 3239-3249.</p>
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Genetic Toxicity - *in vitro*

<p><u>Test Substance</u> <i>Test substance</i></p>	<p>EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538⁰C.</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>Standard method per Ames <i>et al.</i> 1975, 1983 Reverse mutation bacterial assay Salmonella typhimurium with and without metabolic activation Yes. 1984 (1995 publication) Salmonella typhimurium TA100, TA98 Yes Sprague-Dawley rat or Syrian Golden hamster liver (S9 fraction), sex not specified 50ul S9 fraction/plate (0.1ml S9 fraction/1.0ml S9 mix) Aroclor 1254 induced; dosage and treatment not specified 0.0, 0.1, 1.0, 10.0, 50, 100, 500µg/ml ±S9, diluted in dimethyl sulfoxide (DMSO); additional doses with Tween 80 dispersant were 1000, 10,000µg/ml. Mutation induction curve based on revertants/plate was plotted and mutagenic potency, the slope of revertants/µg dose of each induction curve was determined by non-linear regression analysis</p>
<p><u>Test Conditions</u></p>	<p>Assays were conducted only in strains TA 100 and TA98 ±S9, the strains potentially most sensitive to detect activity by this type of complex hydrocarbon. Positive results were observed only in TA98+S9 and are the only data reported. Fresh bacterial stocks were exposed to graded doses of test material ±S9 from rat or hamster liver S9 (3 plate/dose/ liver fraction). Positive control was benzo(a)pyrene (5µg/plate); vehicle controls were DMSO or Tween 80. When detergent dispersant was utilized, equal volumes of test material and Tween 80 were mixed, distilled water was added dropwise to produce emulsions with final concentration of 10%(v/v) oil. Additional dilutions required for testing were made with 10% solutions of Tween 80 in distilled water to maintain constant detergent concentrations in test samples. Aliquots of these dilutions were plated with TA100 and TA98 ±S9 from rat or hamster liver. After 72</p>

<p><u>Results</u> Genotoxic effects</p> <p><u>Conclusions</u> (study authors)</p> <p><u>Data Quality</u> Reliability</p> <p><u>Reference</u></p> <p><u>Other</u> Last changed</p>	<p>hr incubation at 37⁰C, revertant colonies were counted with a Biotran III automatic colony counter.</p> <p>Fuel Oil blend produced a dose related increase in revertant frequency under all testing conditions in strain TA98+S9. Mutagenic potency was enhanced when hamster S9 was employed vs rat S9 (7.22 vs 5.29 revertants/μg, respectively). Detergent dispersion did not increase assay sensitivity for the fuel oil blend. Positive and negative controls performed appropriately; hamster S9 activation improved response of bacteria to benzo(a)pyrene but addition of dispersant did not enhance response beyond hamster S9 alone.</p> <p>EDS fuel oil blend was the most active bacterial mutagen of the high boiling coal liquids tested in this assay and contained the highest level of nitrogen. These positive results agree qualitatively with positive results in dermal carcinogenesis studies; however the lack of quantitative agreement may be related to the fact that the Salmonella assay is highly sensitive to aromatic amines and nitroaromatic compounds while dermal carcinogenesis is predominantly associated with neutral polycyclic aromatic hydrocarbons. Coal derived liquids containing substantial amounts of materials boiling above 370⁰C are active in carcinogenesis screening assays.</p> <p>1. Reliable without restrictions.</p> <p>McKee, R.H., Traul, K.A., and Przygoda, R.T. 1995. Evaluation of coal liquids derived from EDS process in carcinogenesis screening tests. J. Appl. Toxicol. 15: 159-165. (see separate summary for cell transformation data)</p> <p>Ames, B.N. <i>et al.</i> 1975. Mutat. Res. 31: 347-364 Maron, D.M. and Ames, B.N. 1983. Mutat Res. 113: 173-215.</p> <p>14-Dec-01 (Prepared by a contractor to the Olefins Panel).</p>
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Genetic Toxicity - *in vitro*

<u>Test Substance</u>	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C.
<u>Method</u>	
Method/guideline followed	Standard method per Pienta et al, 1977; Przygoda et al, 1985
Type	Cell transformation
System of testing	Syrian Hamster embryo cells (SHE)
GLP	Not specified
Year	1984 (1995 publication).
Species/Strain	Cells isolated from eviscerated, decapitated day 13 embryos of timed-pregnant Syrian hamsters from Eagle Laboratories, Farmersburg, IN, USA.
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	0.0, 0.1, 0.5, 1.0, 5.0, 10.0, 50, 100, 500, 1000µg/ml, diluted in dimethyl sulfoxide (DMSO)
Statistical Methods	None
<u>Test Conditions</u>	Primary cultures of freshly derived hamster embryo cells were grown on Dulbecco's modified Eagles medium with 20% fetal bovine serum for 2 days, then frozen. Each assay consisted of concurrent tests in two distinct pools of embryo cells (each pool comprised of all cells from a single litter). Cell pools were screened with mutagenic and non-mutagenic compounds for validation prior to study initiation. Irradiated feeder cells were plated in 60 mm culture dishes; 24 hr later, non-irradiated target cells were plated at density of 300 cells/dish. One day later, test material was added to 3 plates/concentration/cell pool. Cells were grown for 7 days, then fixed and stained, and transformed colonies identified and counted. Total colony count and number of transformed colonies were recorded for each plate. Cloning efficiency and transforming efficiency were calculated. Assay was valid only if spontaneous and vehicle control cultures had no spontaneous transformed foci and the positive control, benzo(a)pyrene induced transformation at 2 doses (5,

<p><u>Results</u> Genotoxic effects</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliability</p> <p><u>References</u></p>	<p>10µg/ml).</p> <p>Morphological transformation in SHE cells was induced over a concentration range of 1-100µg/ml and was toxic to concentrations exceeding 100µg/ml. Results were comparable in both cell pools. Positive and negative controls performed appropriately.</p> <p>EDS experimental fuel oil blend induced transformation in SHE cells with approximately equal efficiency compared to two other coal derived high boiling liquids tested concurrently. Both neutral PAH and nitrogen-containing PAH fractions are active in this assay. The results of this assay agree with positive dermal carcinogenicity studies and demonstrate that coal derived liquids containing substantial amounts of materials boiling above 370⁰C are active in carcinogenesis screening assays.</p> <p>1. Reliable without restriction.</p> <p>McKee, R.H., Traul, K.A., and Przygoda, R.T. 1995. Evaluation of coal liquids derived from EDS process in carcinogenesis screening tests. <i>J. Appl. Toxicol.</i> 15: 159-165.</p> <p>Pienta, J.A. <i>et al.</i> 1977. <i>Int. J. Cancer</i> 19: 642-655.</p> <p>Przygoda, R.T. <i>et al.</i> 1985. <i>In Vitro</i> 21: 32-38.</p>
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Genetic Toxicity - *in vitro*

<u>Test Substance</u> <i>Test substance</i>	Biphenyl Feedstock, CAS #68989-41-3. Amber-colored liquid with aromatic odor. Compositional analysis, purity and stability referred to sponsor.
<u>Method</u> Method/guideline followed	Standard method based on Hsie et al. (1981), O'Neill & Hsie (1979)
Type	In vitro mammalian cell forward mutation
System of testing	Chinese hamster ovary (CHO) cell culture
GLP	Yes
Year	1984
Species/Strain	CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGPRT+/-) from Oak Ridge National Laboratory, TN.
Metabolic activation	Yes
Species and cell type	Rat liver (S9) fraction purchased from Litton Bionetics, Kensington, MD
Quantity	1.0mg S9 fraction/ml treatment medium
Induced or not induced	Aroclor 1254 induced (treatment not specified)
Concentrations tested	Cytotoxicity: 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048µg/ml ± S9; Mutagenicity: 4, 8, 16, 21, 26, 32, 64µg/ml ±S9; all diluted in 10% Pluronic [®] polyol F68 (prepared in deionized water, mol. wt. 8350).
Statistical Methods	Frequency of mutant colonies per million clonable cells, corrected for absolute survival by viability plates, was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979) using the MUTANT computer program (Snee et al., 1981). Criteria for positive results were a significant (p<0.05) increase in mutant colonies (HGPRT+/- → HGPRT-/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal.
<u>Test Conditions</u>	Sufficient Biphenyl feedstock was weighed separately for each dose level into 10ml volumetric flasks and stored overnight. The following day, flasks were placed in a water bath heated to approx. 93 ⁰ C to melt the biphenyl feedstock, then 7.0ml of 10% F68 was added to the highest dose flask, and to each subsequent dose preparation and vehicle control flask along with sufficient medium (Ham's F-12 without

<p><u>Results</u> Genotoxic effects</p>	<p>hypoxanthine) to achieve final 10ml volume for testing. All flasks were replaced in the water bath. Dosing preparations were vortexed after addition of all components, and just prior to dosing when 20µl were added to each 3 ml culture. All cultures were incubated at 37⁰C in 5% CO₂ enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for -S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For range finding (cytotoxicity), each dose group was composed of 2 flasks, one -S9, one+S9, negative controls ± S9, seeded with 5x10⁵ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10⁶ cells were exposed to biphenyl feedstock for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10⁵-10⁶ cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and 2x10⁵ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10⁻⁵M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17 when they were fixed and stained. For mutagenicity, a ratio of total colony counts in mutagenicity plates over absolute survival in viability plates was calculated for each treatment group. Frequency of mutant colonies/million clonable cells was calculated and statistical comparisons with negative control data were made.</p> <p>In the cytotoxicity test, Biphenyl feedstock induced toxic effects at concentrations of 32 µg/ml and higher -S9, and at 16µg/ml and higher +S9. Relative survival after treatment –</p>
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Genetic Toxicity - *in vitro*

<u>Test Substance</u>	Biphenyl Feedstock, CAS #68989-41-3. Amber liquid with aromatic odor. Composition analysis, purity and stability referred to sponsor.
<u>Method</u>	Standard method based on Williams et al. (1977,1982)
Method/guideline followed	In vitro mammalian cell DNA repair assay
Type	Unscheduled DNA Synthesis (UDS) in primary hepatocyte
System of testing	cultures.
GLP	Yes
Year	1984
Species/Strain	Fischer 344 male rat (11-12 wks old) – 1 rat per test
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Range-finding: 4, 8, 16, 32, 64, 128, 256, 512, 1024,
	2048µg/ml: UDS assay 5.0, 20.0, 50.0, 100µg/ml; all
	diluted in 10% Pluronic [®] polyol F68 (prepared in deionized
	water, mol. wt 8350, 80% hydrophilic)
Exposure period	18.25-18.5 hours
Statistical Methods	None employed. Criteria for positive response are
	incorporation of radioactive precursor (³ H-thymidine) in
	cells that are not normally synthesizing DNA, indicating
	repair of damage. A positive response is defined as a mean
	net nuclear grain count at any treatment level that exceeds
	concurrent negative control by at least 6 grains/nucleus;
	negative control value must not exceed 5 grains. A positive
	response need not be dose related.

<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>F68 control cultures), then fixed in formalin and stained with trypan blue for viability determination. At least 50% viability needed for the assay. In the UDS assay, 1×10^5 cells/ml were seeded into coverslip cultures, exposed to ^3H-thymidine and test substance for 18.25 hours (3 cultures/dose level). Positive control was 2-acetyl aminofluorene (2-AAF, $0.2 \mu\text{g/ml}$). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at $2-8^\circ\text{C}$. Autoradiographs were developed, stained and coverslipped on day 17. Number of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count \div 50) and mean net nuclear grain count (avg. net nuclear grain count/slide \div 3) were calculated. Test slides with negative average net nuclear grain counts were scored as zero.</p> <p>Biphenyl feedstock induced toxicity in primary hepatocytes following 18.5hrs exposure beginning at $8 \mu\text{g/ml}$ (84.3% relative viability) with continuing decreases in viability with increasing dose to the maximum dose of $2048 \mu\text{g/ml}$ (15% relative viability). Toxicity in the UDS assay occurred in the 50 and $100 \mu\text{g/ml}$ dose groups resulting in fewer than 150 viable cells available for counting in each of these groups. Despite this toxicity, a positive, dose-related response for UDS was obtained at all dose levels. Mean net nuclear grain counts were 0.77, 0.00, 15.12, 68.46, 78.42, 251.91, and 248.28 for untreated medium control, vehicle F68 control, 5, 20, 50, $100 \mu\text{g/ml}$ biphenyl feedstock, and 2-AAF positive control, respectively.</p> <p>Biphenyl feedstock induced dose related unscheduled DNA synthesis in cultured rat hepatocytes at all doses evaluated. Biphenyl feedstock causes DNA damage and excision repair in this assay.</p> <p>1. Reliable without restrictions. Study conforms to standard design. GLPs have been followed.</p> <p>Brecher, S., Goode, J.W. 1984. Hepatocyte primary culture/DNA repair test of Biphenyl feedstock. Proj. #2078.</p>
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	<p>Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA.</p> <p>Williams, G.M. 1977. <i>Cancer Res.</i> 37: 1845-1851</p> <p>Williams et al. 1977. <i>In Vitro</i> 13: 809-817</p> <p>Williams et al. 1982. <i>Mut. Res.</i> 97:359-370</p>
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Genetic Toxicity - *in vitro*

<u>Test Substance</u>	Biphenyl Feedstock, CAS #68989-41-3. Amber-colored liquid with aromatic odor. Compositional analysis, purity and stability referred to sponsor.
<u>Method</u>	
Method/guideline followed	Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973)
Type	In vitro cell transformation
System of testing	Mouse embryo cells
GLP	Yes
Year	1983
Species/Strain	BALB/3T3-A31-1-1 from T. Kakunaga, National Cancer Inst., 1982
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Cytotoxicity: 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048µg/ml; Transformation: 4, 8, 16, 32µg/ml, all diluted in 10% Pluronic [®] polyol F68 (prepared in deionized water, mol. wt. 8350, 80% hydrophilic).
Exposure period	2 days
Statistical Methods	None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose.
<u>Test Conditions</u>	Sufficient Biphenyl feedstock was weighed separately for each dose level and melted in an 85 ⁰ C water bath; 0.78ml of 10% F68 added per ml of final volume and balanced salt solution was added as required to achieve final volume for testing. Test preparations were stored at 50-60 ⁰ C until just prior to dosing when the preparations were mixed and added at 50µl to each 5 ml culture. All cultures were incubated at 37 ⁰ C in 5% CO2 enriched humidified atmosphere. For cytotoxicity, 2 flask cultures/dose group, 2 cultures for vehicle F68 or medium negative control were seeded with 1x10 ⁴ cells/culture in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day

<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>4 for at least 20% survival. For transformation, 15 flask cultures (1×10^4 cells/culture/dose group) and two colony formation flask cultures (100 cells/culture/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For transformation cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene ($1 \mu\text{g/ml}$). Colony formation cultures were fixed, stained, and counted visually on day 8 to determine cloning efficiency (avg. number colonies/flask \div 100 cells seeded). Transformation cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci \div total flasks/dose group.</p> <p>Biphenyl feedstock induced toxicity in BALB/3T3 cells after two days exposure beginning at $16 \mu\text{g/ml}$ (49% viability); inducing reduction to 20% viability between 32-$64 \mu\text{g/ml}$ and 2.1% viability at $2048 \mu\text{g/ml}$. In the transformation assay, a progressive increase in cytotoxicity occurred with increasing doses from 8-$32 \mu\text{g/ml}$ reducing the relative cloning efficiency (rel. C.E.) from 72.5-27.5%, respectively. At $32 \mu\text{g/ml}$, the toxic response of 27.5% rel. C.L. was comparable to that of the positive control, 3-methylcholanthrene (28.4% rel. C.E.). The positive control induced the expected response for transformation: 10 type III foci. The vehicle control had 1 type III focus, but the untreated medium control was slightly higher with 2 type III foci. The $8 \mu\text{g/ml}$ and $32 \mu\text{g/ml}$ biphenyl feedstock cultures each had 2 type III foci compared with one type III focus in the vehicle control. However, since the untreated control also had 2 type III foci, the results from treated cultures were considered negative.</p> <p>Biphenyl feedstock did not induce significant transformation in BALB/3T3 cells under conditions of this assay.</p> <p>1. Reliable without restriction.</p> <p>Brecher, S, and Goode, J.W. 1983. BALB/3t3 transformation test: Biphenyl Feedstock. Proj. #2079. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX, USA.</p>
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	<p>Cortesi, E. et al. 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110.</p> <p>Dunkel, V.A. et al. 1981. J. Nat'l Cancer Inst. 67: 1303-1315.</p> <p>Reznikoff, C.A. et al. 1973. Cancer Res. 3239-3249.</p>
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Genetic Toxicity - *in vitro*

<u>Test Substance</u>	Aromatic Pyrolysis Oil, Gulf. CAS #64742-90-1. Brown-black, highly viscous tarry residue. Composition analysis, purity and stability referred to sponsor.
<u>Method</u>	
Method/guideline followed	Standard method based on Hsie et al. (1981), O'Neill & Hsie (1979)
Type	In vitro mammalian cell forward mutation
System of testing	Chinese hamster ovary (CHO) cell culture
GLP	Yes
Year	1984
Species/Strain	CHO-K-1 heterozygous for hypoxanthine-guanine phosphoribosyl transferase (HGPRT+/-) from Oak Ridge National Laboratory, TN.
Metabolic activation	Yes
Species and cell type	Rat liver (S9) fraction purchased from Litton Bionetics, Kensington, MD
Quantity	1.0mg S9 fraction/ml treatment medium (0.3ml S9 fraction in 3 ml medium/flask)
Induced or not induced	Aroclor 1254 induced (treatment not specified)
Concentrations tested	Cytotoxicity: 8, 16, 32, 64, 128, 256, 512, 1023, 2048, 5000µg/ml ± S9; Mutagenicity: 32, 64, 96, 128, 175, 256µg/ml -S9; 128, 175, 256, 375, 512, 750µg/ml +S9; repeat : 500, 600, 750 µg/ml + S9 all diluted in 50% Pluronic [®] polyol F127 (prepared in absolute ethanol, mol. wt. 12,500).
Statistical Methods	Frequency of mutant colonies per million clonable cells was calculated and comparisons of treated cultures with vehicle controls made on transformed data using a two-tailed t-test (Irr & Snee, 1979). Criteria for positive results were significant (p<0.05) increase in mutant colonies (HGPRT+/- → HGPRT-/-) at any dose level and a dose related response. If only one criterion is met, results are considered equivocal.
<u>Test Conditions</u>	Sufficient Aromatic Pyrolysis Oil (APO) was weighed separately for each dose level into 10 ml graduated vials; aliquots were kept under an inert nitrogen blanket overnight. 2.5ml of 50% F127 was added to each treatment vial and vehicle control and medium (Ham's F-12 without hypoxanthine) added as required to achieve final 10ml volume for testing. All dosing preparations were

<p><u>Results</u> Genotoxic effects</p>	<p>thoroughly blended with mixing rods to produce a uniform emulsion, kept in a water bath at 37⁰C until dosing and mixed again just prior to use when 20µl of each preparation was added to 3ml treatment medium/culture vessel. All cultures were incubated at 37⁰C in 5% CO₂ enriched, humidified atmosphere. Positive control mutagens were ethyl methanesulfonate (100µg/ml) for -S9 cultures, and benzo(a)pyrene (4µg/ml) for +S9 cultures. For cytotoxicity, each dose group was composed of 2 flasks, one -S9, one+S9, negative controls ± S9, seeded with 5x10⁵ cells on day 1. Cultures were exposed to test compound for 5 hours on day 2. On day 3, cells were trypsinized and counted with a Coulter Model ZB, then 200 cells were transferred into each of 3 60mm culture dishes. These viability plates were incubated until day 10, fixed in methanol and stained with Giemsa. Colonies were counted visually or with an Artek Model 981 colony counter. Absolute survival = total colony count ÷ number of cells seeded/flask. Relative survival = absolute survival in treated cultures ÷ vehicle control survival. Acceptable survival level is at least 10%. For mutagenicity, cells were seeded on day 1 into 6 flasks/dose group, 3-S9, 3+S9; on day 2 approximately 10⁶ cells were exposed to APO for 5 hours. Vehicle control had 12 flasks, 6-S9, 6+S9. On day 3, cultures with excessive cytotoxicity were discarded. From remaining cultures, 200 cells were seeded to each of 4 viability plates/dose level; incubated to day 10, fixed with methanol, stained with Giemsa, and colonies counted for survival. Expression cultures (10⁵-10⁶ cells/one dish/dose) were seeded on day 3; subcultured three times until day 10 when 200 cells were seeded on each of 4 viability plates/dose and 2x10⁵ cells seeded on each of 5 mutagenicity plates/dose with selective medium containing 10⁻⁵M 6-thioguanine to allow expression of HGPRT mutation. Cultures were incubated undisturbed until day 17-18 when they were fixed and stained. For mutagenicity, a ratio of total colony counts in mutagenicity plates over absolute survival in viability plates was calculated for each treatment group. Frequency of mutant colonies/million clonable cells was calculated and statistical comparisons with negative control data were made.</p> <p>In the cytotoxicity test, APO induced cell toxicity beginning at 32µg/ml +S9 (cells x10⁵/ml 5.0, 4.3, 3.8, 3.9, 2.5 and 2.3 at 0[vehicle control], 32, 64, 128, 256 and 512µg/ml) and at</p>
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	<p>Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA.</p> <p>Hsie, A.W. et al. 1981. Mut. Res. 86: 193-214.</p> <p>O'Neill, J.P. and Hsie, A.W. 1979. Banbury Report 2: 55-63.</p> <p>Irr, J.D. and Snee, R.D. 1979. Banbury Report 2: 263-275.</p>
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Genetic Toxicity - *in vitro*

<u>Test Substance</u>	Aromatic Pyrolysis Oil, Gulf. CAS #64742-90-1. brown-black, highly viscous tarry residue. Composition analysis, purity and stability referred to sponsor.
<u>Method</u>	Standard method based on Williams et al. (1977,1982)
Method/guideline followed	In vitro mammalian cell DNA repair assay
Type	Unscheduled DNA Synthesis (UDS) in primary hepatocyte
System of testing	cultures.
GLP	Yes
Year	1984
Species/Strain	Fischer 344 male rat (9-10 wks old) – 1 rat per test
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Range-finding: 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/ml:
	UDS assay 0.5, 2.0, 10, 60µg/ml; all diluted in 50%
	Pluronic [®] polyol F127 (prepared in absolute ethanol, mol.
	wt 12,000, 70% hydrophilic)
Exposure period	18-20 hours
Statistical Methods	None employed. Criteria for positive response are
	incorporation of radioactive precursor (³ H-thymidine) in
	cells that are not normally synthesizing DNA, indicating
	repair of damage. A positive response is defined as a mean
	net nuclear grain count at any treatment level that exceeds
	concurrent negative control by at least 6 grains/nucleus;
	negative control value must not exceed 5 grains. A positive
	response need not be dose related.
<u>Test Conditions</u>	Sufficient Aromatic Pyrolysis Oil (APO) was weighed
	separately for each dose level, 0.75ml of 50% F127 added
	per ml of final volume and sufficient medium (Williams
	Medium E with 10% fetal bovine serum and insulin) added
	to achieve final volume. Test preparations were mixed just
	prior to addition at 30 or 50µl to each 3 or 5ml culture,
	respectively. The conc. of ³ H-thymidine (½ life 12.5 yrs.)
	used in these assays was 1mCi/ml. All cultures were
	incubated at 37 ⁰ C in 5% CO ₂ enriched humidified
	atmosphere. For range-finding, primary hepatocytes
	derived from freshly perfused rat liver were seeded (approx.
	1x10 ⁵ cells/ml) into treatment vessels, exposed to test
	material for 18 hours (2 cultures/dose level; 2 untreated

<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>cultures, and two vehicle F127 control cultures), then fixed in formalin and stained with trypan blue for viability determination. At least 50% viability needed for the assay. In the UDS assay, 1×10^5 cells/ml were seeded into coverslip cultures, exposed to ^3H-thymidine and test substance for 18 hours (3 cultures/dose level). Positive control was 2-acetyl aminofluorene (0.2 $\mu\text{g/ml}$). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at 2-8$^{\circ}\text{C}$. Autoradiographs were developed, stained and coverslipped on day 14. Number of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count \div 50) and mean net nuclear grain count (avg. net nuclear grain count/slide \div 3) were calculated.</p> <p>APO induced toxicity in primary hepatocytes beginning at 4 $\mu\text{g/ml}$ (51.2% viability) following 18-20 hours exposure. Viability continued to decrease in a generally dose related manner to the maximum dose of 1024 $\mu\text{g/ml}$ (0.8% relative viability). UDS occurred in a dose related manner, increasing from 117 net nuclear grains at 2 $\mu\text{g/ml}$ to 218 grains at 60 $\mu\text{g/ml}$ compared to a vehicle control net count of 0.63 and positive control of 363 net nuclear grains.</p> <p>Aromatic Pyrolysis Oil induced dose related unscheduled DNA synthesis in cultured rat hepatocytes. Aromatic Pyrolysis Oil causes DNA damage and repair in this assay.</p> <p>1. Reliable without restrictions. Study conforms to standard design. GLPs have been followed.</p> <p>Brecher, S., Goode, J.W. 1984. Hepatocyte primary culture/DNA repair test of aromatic pyrolysis oil. Proj. #2083. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA.</p> <p>Williams, G.M. 1977. Cancer Res. 37: 1845-1851.</p> <p>Williams <i>et al.</i> 1977. In Vitro 13: 809-817.</p> <p>Williams <i>et al.</i> 1982. Mut. Res. 97:359-370.</p>
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Genetic Toxicity - *in vitro*

<u>Test Substance</u>	Aromatic Pyrolysis Oil, Gulf. CAS #64742-90-1. Dark brown-black, highly viscous tarry residue. Composition analysis, purity and stability referred to sponsor.
<u>Method</u>	
Method/guideline followed	Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973)
Type	In vitro cell transformation
System of testing	Mouse embryo cells
GLP	Yes
Year	1983
Species/Strain	BALB/3T3-A31-1-1 from T. Kakunaga, National Cancer Inst., 1982
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Cytotoxicity: 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/ml; Transformation: 8, 16, 32, 64, 128, 256 µg/ml, all diluted in 50% Pluronic [®] polyol F127 (prepared in absolute ethanol, mol. wt. 12,000, 70% hydrophilic).
Exposure period	2 days
Statistical Methods	None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose.
<u>Test Conditions</u>	Sufficient Aromatic Pyrolysis Oil (APO) was weighed separately for each dose level, 0.75ml of 50% F127 added per ml of final volume and medium (Eagles MEM with 10% heat-inactivated fetal calf serum) added as required to achieve final volume for testing. Test preparations were mixed just prior to addition to cultures at 50µl to each 5 ml culture. All cultures were incubated at 37 ⁰ C in 5% CO ₂ enriched humidified atmosphere. For cytotoxicity, 2 plate cultures/dose group, 2 plate cultures for vehicle F127 or medium negative control were seeded with 1x10 ⁴ cells/plate in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 20% survival. For transformation, 15 flasks (1x10 ⁴ cells/flask/dose group)

<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>and two plate cultures (100 cells/plate/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For flask cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1ug/ml). Plate cultures were fixed, stained, and counted visually on day 9 to determine cloning efficiency (avg. number colonies/plate ÷ 100 cells seeded). Flask cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group.</p> <p>APO induced toxicity in BALB/3T3 cells after two days exposure beginning at 128 µg/ml (59% relative survival); maximum toxicity (80%) occurred between 256-512 µg/ml and plateaued at 1024 µg/ml. APO induced transformed foci at 128, 256 µg/ml, the two highest dose levels, with borderline positives but inconsistent responses at 8-64 µg/ml. Compounds that transform cells have a high probability of inducing tumors if injected into immunosuppressed mice. Positive and negative controls gave expected responses.</p> <p>Aromatic Pyrolysis Oil induced transformation in BALB/3T3 cells under conditions of this assay. Cytotoxicity and impairment of cloning efficiency were also observed at the two highest dose levels.</p> <p>1. Reliable without restriction.</p> <p>Brecher, S., Good, J.W. 1983. BALB/3T3 transformation test: Aromatic Pyrolysis Oil. Proj. #2084. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX, USA.</p> <p>Cortesi, E. <i>et al.</i> 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110.</p> <p>Dunkel, V.A. <i>et al.</i> 1981. J. Nat'l Cancer Inst. 67: 1303-1315.</p> <p>Reznikoff, C.A. <i>et al.</i> 1973. Cancer Res. 3239-3249.</p>
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Genetic Toxicity - *in vitro*

<u>Test Substance</u>	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
<u>Method</u>	
Method/guideline followed	Standard method per Ames et al, 1975
Type	Reverse mutation bacterial assay
System of testing	Salmonella typhimurium with and without metabolic activation, Sacchromyces cerevisiae
GLP	Yes
Year	1977
Species/Strain	S. typhimurium TA1535, TA1537, TA1538, TA100, TA98; S. cerevisiae D4
Metabolic activation	Yes
Species and cell type	Sprague Dawley adult male rat liver (S9 fraction)
Quantity	50µl S9 fraction in 0.5ml S9 mix/plate
Induced or not induced	Aroclor 1254 induced, administered to male rats 5 days prior to sacrifice
Concentrations tested	0, 0.001, 0.01, 0.10, 1.0 and 5.0µl/plate ± S9; diluted in dimethyl sulfoxide (DMSO); negative control 50 µl DMSO/plate
Statistical Methods	None specified. Positive response criteria per Ames et al., (1975) were number of mutant colonies equal to or greater than 2 –3 times reversion frequency of negative control for each strain. Results must be reproducible in a repeat assay.
<u>Test Conditions</u>	Salmonella strains or S. cerevisiae D4 (approx. 10 ⁸ /ml) were added to separate test tubes containing 2.0ml molten top agar and appropriate doses of RTB were administered to each tube. Just prior to pouring, 0.5ml S-9 mix was added to tubes receiving metabolic activation and contents was poured on minimal agar plates. Number of tubes or plates ±S9 was not reported. Plates were incubated for 48 hrs at 37 ⁰ C and scored for number of colonies on each plate. One complete assay was performed with all strains. Repeat assays of D4 -S9, and TA1538, TA 98+S9 were performed to evaluate results from the 1 st assay. Positive control compounds were: 2-nitrofluorene (NF, 100µg/plate) for TA98, TA1538 –S9; methylnitrosoguanidine (MNNG, 10µg/plate) for TA1535,

<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>TA100, D4 -S9; quinacrine mustard (QM, 10µg/plate) for TA1537 -S9 and 2-anthramine (ANTH, 100µg/plate) for TA1535, TA100 +S9, dimethylnitrosamine (DMNA, 100µM/plate) for D4 +S9, 2-acetylaminofluorene (AAF, 100µg/plate) for TA1538, TA98 +S9, and 8-aminoquinoline (AMQ, 100µg/plate) for TA1537+S9.</p> <p>Rerun tower bottoms was toxic to Salmonella strains TA1535, TA1537, TA98 and S. cerevisiae D4 at 5.0µl/plate. Test material did not increase revertant frequency in any Salmonella strain without metabolic activation. Test of D4 was repeated at doses of 1.0 and 5.0µl/plate due to slight increased revertant frequency at 1.0µl/plate in the 1st test and toxicity at 5.0µl/plate. No increase in revertants was observed at 1.0µl and toxicity persisted at 5.0µl in the repeat assay. In the presence of S9 metabolic activation, no increase in revertant frequency was seen in TA1535, TA1537, TA100 and D4 in the first assay. A repeat test with TA98 and TA100 was performed at 1.0 and 5.0µl/plate because these strains exhibited a dose-related increase in revertant frequency [TA98+ S9: incidence of revertants was 25, 22, 33; TA1538+ S9, incidence of revertants was 26, 48, 88 at negative control, 1.0 and 5.0µl/plate, respectively]. Repeat assay was considered negative [TA98+ S9: incidence of revertants was 55, 57, 99; TA1538+ S9, incidence of revertants was 27, 44, 33 at negative control, 1.0 and 5.0µl/plate, respectively]</p> <p>Rerun tower bottoms did not demonstrate reproducible mutagenic activity in any of the assays and was not considered to be mutagenic under these test conditions.</p> <p>2. Reliable with restrictions. Report did not indicate number of tubes or plates used per dose. Criteria for a positive response were not defined in the report.</p> <p>Brusick, D.J. 1977. Mutagenicity evaluation of MCTR-36-77. LBI Proj #2683. Litton Bionetics, Inc. Kensington, MD for Mobil Chemical Co., Edison, NJ, USA.</p> <p>Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ, USA.</p>
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	Ames, B.N., McCann, J., Yamasaki, E. 1975. Mutat. Res. 31: 347-364.
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Genetic Toxicity - *in vitro*

<u>Test Substance</u>	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
<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	Standard method, no guideline specified Sister chromatid exchange (SCE) Human lymphocytes Yes 1981 Human venous blood, primary culture No NA NA NA 1 st trial : 0, 0.0003, 0.001, 0.003, 0.011, 0.033, 0.111, 0.333, 1.1, and 3.3µl/ml 2 nd trial: 0. 0.02, 0.04, 0.06, 0.08, and 0.10µl/ml, diluted in dimethyl sulfoxide (DMSO). Negative control was 1% DMSO (100µl/culture).
Statistical Method	Students t-test to compare SCE frequency in treated cultures with negative controls. Positive results require approximate doubling in SCE frequency over background levels at a minimum of 3 doses. In the absence of doubling, positive response requires a statistically significant increase at a minimum of 3 doses and a positive dose response.
<u>Test Conditions</u>	Human venous blood was drawn into a sterile heparinized syringe; cultures were initiated by adding 0.6ml blood to 9.4ml RPMI 1640 medium supplemented with fetal calf serum, 1% antibiotics and 5-bromodeoxyuridine (BrdU, 25µM), and incubated at 37 ⁰ C in the dark for 24 hours. Dilutions of test material, DMSO or positive control compound, ethyl methane sulfonate (EMS, 0.1µl/ml) were added to cultures (2 cultures/ dose) and incubation continued for 46-50hrs. Colcemid was added 2-3hrs, prior to harvest, to arrest dividing lymphocytes in metaphase. After growing for 2 cell cycles in the presence of BrdU, chromosomes were fixed and stained using fluorescence (5µg/ml Hoechst 33258) with exposure to black light (15-20 min) and 5% Giemsa for an additional 10-20 min, so that

<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>one chromatid is dark and its pair or “sister” is pale, allowing exchanged segments between them to be clearly visible. Fifty cells in M2 (2nd division metaphase)/dose were scored for frequency of SCE/cell and per chromosome. All slides were coded prior to scoring and scored “blind”</p> <p>In the first trial, no scorable metaphases were found at 3.3µl/ml; at 1.11µl/ml, 50 metaphases could not be found due to cell cycle delay and a reduction in mitotic index. However, there was a statistically significant increase in SCE at 1.11µl/ml and 0.33µl/ml (13.8 and 11.1 SCE/cell respectively, compared to 7.0 SCE/cell in DMSO controls). In the 2nd trial, cultures were treated at concentrations of 0.02-0.10µl/ml and incubated for an additional 4 hours to allow cells to reach second metaphase. Pronounced cell cycle delay occurred at 0.08 and 0.10µl/ml. There were statistically significant increases in SCE/cell at 0.06 (15.1 SCE/cell), 0.08 (16.1 SCE/cell) and 0.10µl/ml (13.1 SCE/cell) compared to DMSO negative control (10.6 SCE/cell) with some evidence of a dose response. Positive control compound. EMS, induced approximately 33 SCE/cell in both assays. Doubling of SCE incidence over DMSO controls was not reached. While the SCE increase was not large, it was apparently dose related and the authors considered this effect to be a weak positive response.</p> <p>A small but statistically significant increase in sister chromatid exchange was demonstrated in human lymphocytes treated with RTB. Rerun tower bottoms was considered to show a weakly positive response under conditions of this assay.</p> <p>1. Reliable without restrictions</p> <p>Galloway, S.M. 1981. Mutagenicity evaluation of 081088003 in the sister chromatid exchange assay in human lymphocytes. Assay #5634. Litton Bionetics, Inc., Kensington, MD for Mobil Oil Corp, (Study # 1711-80) Princeon, NJ, USA.</p> <p>Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co.,</p>
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	Princeton, NJ, USA.
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Genetic Toxicity - *in vivo*

<p><u>Test Substance</u> Remarks</p>	<p>Light Pyrolysis fuel oil, Gulf CAS #68527-18-4 Water white liquid. Compositional analysis, purity and stability referred to sponsor.</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>Comparable to standard assay Mammalian bone marrow erythrocyte micronucleus Yes 1984 Mouse Crl:CD[®]-1 (ICR) BR Swiss Male and female: Range finding (RF) 2M, 2F/group; Micronucleus 10M, 10F/group; 15M, 15 F in 1 group (11-12wks old at study initiation). Oral gavage RF:0, 1.25, 2.5, 5.0 g/kg in corn oil; Micronucleus: 0, 0.25, 0.5, 1.0 g/kg 1 dose/day for 2 days; 1 group- 1 dose, 1 day only Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant (p<0.05) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.</p>
<p><u>Test Conditions</u></p>	<p>Light pyrolysis fuel oil (LPFO) dosing solutions were prepared fresh for each day of dosing – for RF 2.5 g LPFO mixed with corn oil to make 10 ml; for micronucleus 2.5 g LPFO mixed with corn oil to make 50 ml, blended by shaking. Based on results of range finding study, three groups of mice were given LPFO by oral gavage daily for two days. All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 1.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with</p>

<p><u>Results</u> Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>References</u></p>	<p>May-Grunewald /Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.</p> <p>In the range finding study, all mice given LPFO at 5.0 or 2.5 g/kg died by day 3. At 1.25 g/kg, ½ females died, surviving mouse was lethargic, males appeared normal. In micronucleus test, 1/10 males given 1.0 g/kg (2 doses) died by day 3. No other mortality or significant weight changes were observed. Surviving mice treated with LPFO did not show any significant changes in micronucleus formation in PCE and no significant changes in the ratio of PCE/NORM compared to vehicle controls.. NOEL (genetic)= 1.0 g/kg. NOEL (systemic)= 0.5g/kg</p> <p>Oral treatment of mice with Light Pyrolysis Fuel Oil for 1-2 days at doses up to 1.0 g/kg/day had no effect on frequency of micronucleated polychromatic erythrocytes in bone marrow. Under these test conditions LPFO does not induce cytogenetic damage.</p> <p>1. Reliable without restrictions.</p> <p>Khan, S.H. 1984. Micronucleus test of Light Pyrolysis Fuel Oil. Proj. #2106. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Genetic Toxicity - *in vivo*

<u>Test Substance</u>	Biphenyl Feedstock, CAS #68989-41-3. Amber-colored liquid with aromatic odor. Compositional analysis, purity and stability referred to sponsor.
<u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period Statistical methods	Comparable to standard assay Mammalian bone marrow erythrocyte micronucleus Yes 1984 Mouse Crl:CD [®] -1 (ICR) BR Swiss Male and female: 10M, 10F/group; 15M, 15 F in 1 group Oral gavage 0, 0.25, 0.5, 1.0g/kg in corn oil 1 dose/day for 2 days; 1 group- 1 dose, 1 day only Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant (p<0.05) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.
<u>Test Conditions</u>	Biphenyl feedstock dosing solutions were prepared fresh for each day of dosing –2.5 g was mixed with corn oil to make 50 ml, blended by shaking. In an initial study at doses of 0.0, 0.75, 1.5, 2.0, and 3.0g/kg, very high mortality occurred at doses of 1.5-3.0g/kg and the study was terminated. A new study was initiated at doses of 0.25 –1.0g/kg in a single dose daily by gavage for 2 days All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 1.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald /Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were

<p><u>Results</u> Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>References</u></p>	<p>counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.</p> <p>No mortality occurred at any dose level and no effects on body weight were observed in either sex. Mice treated with biphenyl feedstock did not show any significant change in the frequency of micronucleus formation in PCE and no significant changes in the ratio of PCE/NORM compared to vehicle controls. Positive and negative controls performed appropriately. NOEL(genetic and systemic) = 1.0g/kg</p> <p>Oral treatment of mice with Biphenyl feedstock for 1-2 days at doses up to 1.0 g/kg/day had no effect on frequency of micronucleated polychromatic erythrocytes in bone marrow. Under these test conditions LPFO does not induce cytogenetic damage.</p> <p>1. Reliable without restrictions. Study conforms to standard design. GLP have been followed.</p> <p>Khan, S.H. 1984. Micronucleus test in mouse bone marrow: Biphenyl feedstock administered orally for 2 days. Proj. #2077. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Genetic Toxicity - *in vivo*

<u>Test Substance</u>	Aromatic Pyrolysis Oil, Gulf CAS #64742-90-1 Dark brown - black, highly viscous residue. Compositional analysis, purity and stability referred to sponsor.
<u>Method</u> Method/guideline followed Type GLP Year Species Strain Sex Route of administration Doses/concentration levels Exposure period Statistical methods	Comparable to standard assay Mammalian bone marrow erythrocyte micronucleus Yes 1984 Mouse CrI:CD [®] -1 (ICR) BR Swiss Male and female: 10M, 10F/group; 15M, 15 F in 1 group (11 wks old at initiation) Oral gavage 0.0, 1.25, 2.5, 5.0 g/kg in corn oil 1 dose/day for 2 days; 1 group- 1 dose, 1 day only Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant (p<0.05) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.
<u>Test Conditions</u>	Aromatic pyrolysis oil (APO) dosing solutions were prepared fresh for each day of dosing –12.5 g APO mixed with corn oil to make 50 ml, blended by shaking. Three groups of mice were given APO by oral gavage daily for two days. All mice were weighed on day 1 and on day of sacrifice. One half of each treated group and vehicle control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 5.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald/Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each

<p><u>Results</u> Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>References</u></p>	<p>day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.</p> <p>Males at all dose levels treated with 1.25-5.0 g/kg APO for 2 days showed statistically significant ($p < 0.05$) dose related increases in % micronucleated PCE compared to concurrent negative controls at both day 3 and day 4 sacrifices, except for the 1.25 g/kg group on day 4 which was significantly higher than the historical control only. Females given 2 doses and sacrificed on days 3 and 4 showed statistically significant ($p < 0.05$) increases in % micronucleated PCE compared to negative controls at 5.0 g/kg only. All mice given 1 dose of 5.0 g/kg and sacrificed on day 2, 3, 4 showed positive responses compared to negative controls. There were no significant changes in the ratio of PCE/NORM compared to controls. LOEL (males) = 1.25 g/kg: NOEL (females) = 2.5 g/kg</p> <p>Oral treatment of mice with Aromatic Pyrolysis Oil for 1 or 2 days induced increased frequency of micronucleated polychromatic erythrocytes in bone marrow and is considered positive. APO can induce cytogenetic damage in this test system.</p> <p>1. Reliable without restrictions.</p> <p>Khan, S.H. and Goode, J.W. 1984. Micronucleus test: Aromatic Pyrolysis Oil orally for 2 days. Proj. #2082. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Repeated Dose Toxicity

<u>Test Substance</u> Remarks	Light Pyrolysis Fuel Oil, CAS# 68527-18-4 Water white liquid
<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	No guidelines specified, comparable to standard study. Subacute Yes 1985 Rat F344 Dermal 8 days 0, 1.0, 2.0 g/kg Males and females 6 hr/day once/day for 5 days Light paraffinic oil (2.16 ml/kg) 2 days Analysis of Variance, Dunnett's test
<u>Test Conditions</u>	Rats were housed in suspended stainless cages with wire mesh bottoms and fronts with automatic watering in a room maintained at 72.8 ⁰ F, relative humidity of 56% and 12 hour light/dark cycle. Chow diet and water were provided ad lib. Doses were administered over 10% of body surface to the backs of rats clipped free of hair and fitted with Elizabethan collars to reduce ingestion. After 6 hours, collars were removed and residual oil wiped off. The test substance mixture was analyzed at initiation and termination of dosing period for concentration/stability/uniformity by fluorescence. Observations for mortality and moribundity were made daily, and for clinical signs daily on dosing days. Body weight was recorded at initiation and termination. Necropsy and gross pathology were conducted but histopathology, clinical pathology and organ weights were not obtained.
<u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks	NOEL not determined. LOEL = 1g/kg/day (based on skin irritation). No mortality or morbidity was observed and there were no

<p><u>Conclusions</u></p> <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>test article related clinical signs. By day 5, males and females in both dose groups had well defined erythema, with some resolution and eschar formation by day 8. Slight edema was seen in the high dose males and females that resolved by day 8. A significant decrease in body weight was noted in high dose males.</p> <p>No mortality or morbidity were observed. Group mean body weight was decreased in males in the 2.0 g/kg group. Skin irritation occurred in rats receiving 1.0 and 2.0 g/kg of test article that partially resolved after the 2 day recovery period with severe erythema/eschar formation in 4 rats (3M, 1F) in the high dose group.</p> <p>2. Reliable with restrictions. This study was limited in scope, being restricted to determination of morbidity/mortality, clinical signs including scoring for skin irritation, body weight changes and gross necropsy.</p> <p>Rausina, G. 1985. Five-day repeated dose dermal toxicity study in rats of light pyrolysis fuel oil. Proj. #2109A. Gulf Life Sciences Center, Pittsburgh, PA. For Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Repeated Dose Toxicity

<u>Test Substance</u> Remarks	Light Pyrolysis Fuel Oil, CAS #68527-18-4. No analysis of purity or composition reported, referred to sponsor.
<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	No guidelines specified, comparable to standard study. Subacute Yes 1985 Rat Fischer 344 Whole body Inhalation 4 weeks 0, 0.51, 1.26, 2.54 g/m ³ (actual) Males and females (10/sex/group) 6 hr./ day 5 days/wk. For 4 wks. Filtered air, 6 hrs/day, 5 days/wk. None Analysis of variance, Dunnett's test, Kolmogorov-Smirnov test
<u>Test Conditions</u>	Rats were housed individually in stainless steel, screen-bottomed cages with automatic watering systems in rooms maintained at 72.7 ⁰ F with 59% relative humidity and a 12 hour light/dark cycle. Chow diet and water were provided ad lib except during exposure. Chamber concentrations were monitored by GC; peak areas were compared with those of liquid test article standards. Target dose levels were 0.5, 1.25, and 2.5 g/m ³ administered as an aerosol. (Actual levels cited above). Each rat was observed twice daily on weekdays and once daily on weekends for morbidity and mortality, and once daily (immediately after exposure) for clinical signs. Body weight was taken at initiation and weekly thereafter. Blood was taken from non-fasted rats via the orbital sinus. At study termination, rats were necropsied for gross lesions and organs/tissues (34/rat) were preserved for histopathological evaluation (0, 1.26 and 2.54 g/m ³ groups).
<u>Results</u> NOAEL (NOEL) LOAEL (LOEL)	Not determined LOEL = 0.51 g/m ³ (decreased. body wt, dec. hematocrit,

<p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>1. Reliable without restrictions.</p> <p>Rausina, G. 1985. Four-week repeated dose inhalation toxicity study in rats of Light Pyrolysis Fuel Oil. Proj. #84-2111. Gulf Life Sciences Center, Pittsburgh, PA. For Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Pyrolysis Fuel Oil, (water and oil quenched). Tested as supplied by producer. Produced by pyrolysis at temperatures up to 950 ⁰ C, contained 300-500 ppm benzo(a)pyrene
Remarks	
<u>Method</u>	
Method/guideline followed	No guidelines specified
Test type	Chronic/cancer study – skin painting
GLP	No
Year	1977
Species	Mouse (40/group/dose)
Strain	C3H/HeJ
Route of administration	Dermal
Duration of test	Lifetime (28 mon)
Doses/concentration levels	One brushfull – Mon, Wed., Fri.
Sex	Not specified
Exposure period	Continuous, no test article removal
Frequency of treatment	3 times/wk.
Control group and treatment	Water- 1 brushfull 3 times/wk
Post exposure observation period	None
Statistical methods	Least squares to determine median latent period
<u>Test Conditions</u>	Doses of neat test article or water were brushed on to the backs of mice, clipped free of hair, three times/wk. Doses were applied consistently to the midline but qualitatively, with each dose described as a brushfull. At monthly intervals, papilloma or carcinoma indexes were calculated (100 times the ratio of number of mice with skin tumors divided by the effective group). Effective group was calculated as original number of mice at start divided by the number dead without tumors, but maintained constant after appearance of the median tumor. Median papilloma or carcinoma latent periods were also determined. Lesions were verified by histopathology.
<u>Results</u>	
NOAEL (NOEL)	Not applicable
LOAEL (LOEL)	Not applicable
Remarks	Results of the water-quenched and oil-quenched oils were essentially identical. Both samples were highly carcinogenic. For the water quenched oil, the papilloma and carcinoma indexes were 100 and 97.2, respectively, and

<p><u>Conclusions</u></p> <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p> <p><u>Other</u> Last changed</p>	<p>the median papilloma and cacinoma latent periods were 10.2 and 12.2 months, respectively. For the oil quenched oils, the indexes were 94.4 and 94.4, respectively, and the latent periods 10.3 and 12.1 months. The malignant tumors were squamous call carcinomas.</p> <p>Pyrolysis fuel oil (both water and oil quenched) was carcinogenic in the mouse skin painting bioassay.</p> <p>2. Reliable with restrictions. Although the method used the early qualitative procedure of actual skin painting rather than exact volume application, and the material was not analyzed, the results are unambiguous. The described process conditions and high B(a)P levels are consistent with a dermal carcinogenic response and the latency period was short enough to anticipate possible metastatic spread (Reviewer's comments).</p> <p>Weil, C.S., Condia, N.I. 1977. Experimental carcinogenesis of pyrolysis fuel oil. Am. Ind. Hyg. Assoc. J. 38: 730-733.</p> <p>4/11/2001 (Prepared by a contractor to the Olefins Panel)</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C.
<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	Other 28-day dermal. Yes 1983 (1985 publication) Rabbit New Zealand White Dermal 4 weeks 0, 50, 200 mg/kg Males and females (5/sex/dose) 4 weeks 5 days/wk 5M, 5F; Primol 185 (highly purified mineral oil; white oil) None Dunnett's test for comparing means of test groups and controls
<u>Test Conditions</u>	Rabbits (3-5kg) were individually housed in suspended steel cages in a room maintained between 18-22 ⁰ C, relative humidity of 40-70% and a 12 hr light/dark cycle. Food and water was available ad lib. Test material was applied to unabraded skin in an area of approx. 200cm ² on the dorsal surface between the shoulders and lumber region. Elizabethan collars were used to minimize ingestion. Test material (50 and 200mg/kg) was diluted in Primol 185, and administered at 2ml/kg for 5 consecutive days/wk during the 4 wk dosing period. Residual test article was allowed to remain or accumulate on the skin. The 200mg/kg dose selected was the highest dose that did not cause weight loss in a 5 day range-finding study. Rabbits were clipped twice weekly during the 4 wk dosing period. Rabbits were observed daily for clinical signs of toxicity and skin irritation. Body weight and food consumption were determined weekly. Blood was collected for hematology and clinical chemistry from unfasted rabbits prior to dosing, and at sacrifice (24 hr fasted). All animals were necropsied

<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p> <p><u>Conclusions</u></p>	<p>and organ wt determined for liver, kidney, epididymides and testes. Tissue preserved for histopathological examination included brain, heart, lungs, liver, kidney, spleen, testis, epididymis, prostate, seminal vesicle, ovary, urinary bladder, adrenal, pancreas, thymus, bone marrow, and skin.</p> <p>NOAEL not determined LOAEL = 50mg/kg based on body weight reduction, liver weight increase, serum cholesterol increase (estimated by reviewer).</p> <p>No mortality occurred during the study. At the site of application, desquamation, blanching, atonia and fissuring were observed in the high dose group. The low dose group and controls showed only a low incidence of desquamation. There was a dose-related decrease in mean group body weight of both males and females that became more pronounced over time; however, significance was reached only in the high dose females. Food consumption was also reduced but not to levels of statistical significance. All dosed rabbits showed statistically significant, dose-related increases in liver and kidney weight and organ to body weight ratios. Diffuse hepato-cytomegaly was evident in livers from 9/10 high dose and 4/10 low dose rabbits; effects ranged from slight to severe. Cytoplasmic degeneration and vacuolated hepatocytes were occasionally seen. Thymic atrophy was observed in 6/8 high dose rabbits but not in the low dose group. No other microscopic abnormalities were observed. Blood cholesterol levels were significantly elevated in a dose related manner in both sexes; other clinical chemistry values were within normal ranges.</p> <p>The test substance elicited dermal irritation as well as systemic effects that might have been related to weight loss or stress. Hepatic alterations were manifested as liver enlargement, elevated serum cholesterol, gross observations of liver abnormalities, and microscopic findings of diffuse hepatocytomegaly, cytoplasmic degeneration and hepatocellular vacuolization. Kidney weight and kidney weight to body weight ratio were elevated in both sexes, in a dose responsive manner, but there were no histopathological abnormalities observed.</p>
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<p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>1. Reliable without restrictions</p> <p>McKee, R.H., Kapp, R.W., and Ward, D.P. 1985. Evaluation of the systemic toxicity of coal liquefaction-derived materials following repeated dermal exposure in the rabbit. J. Appl. Toxicol. 6: 345-351.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C.
<u>Method</u>	Other
Method/guideline followed	90-Day oral toxicity.
Test type	Yes
GLP	1984 (1987 publication)
Year	Rat
Species	Sprague-Dawley (Hilltop Laboratory Animals, Scottsdale, PA, 6 wks old at receipt)
Strain	Oral gavage.
Route of administration	Approx. 104 days
Duration of test	0, 0.02, 0.1, 0.5 g/kg/day diluted in highly refined white oil (CAS #8012-95-1)
Doses/concentration levels	Male and female (18M, 18F/dose group)
Sex	13 wks (90 days)
Exposure period	Once a day, 5 days/wk
Frequency of treatment	36M, 18F; 5ml/kg white oil; once a day, 5 days/wk
Control group and treatment	2 weeks
Post exposure observation period	Bartlett's test of homogeneity of variance, standard ANOVA, Duncan's test, linear regression for dose response; Kruskal Wallis (non-parametric test) followed by Dunn's Summed Rank test if appropriate; Jonckheere's test for monotonic trends in dose groups.
Statistical methods	
<u>Test Conditions</u>	This assay was the subchronic toxicity portion of a combined Reproductive/Subchronic toxicity study with an initial group size of 18M, 54F/dose group and 36M, 90F/control. Animals were assigned to 4 treatment groups based on body weight and were housed individually in stainless steel cages in rooms maintained at 20-24 ⁰ C temperature, 40-70% relative humidity and 12 hr light/dark cycle. Animals received water and food ad lib. Test material was administered by gavage 5 times weekly for 13 wks; body weight was recorded prior to dosage initiation (day 0) and weekly throughout the exposure period. At the end of 13 wks of treatment, 18 female rats/group were selected for the subchronic study and the others mated. All males were mated for 10 days. Fourteen days after

<p><u>Results</u> NOAEL (NOEL) Remarks</p> <p><u>Conclusions</u></p> <p><u>Quality</u> Reliability</p>	<p>termination of dosing and following confirmation of mating, blood samples were collected from males and 18 females /group. Animals were killed and necropsied. Brain, kidneys, adrenals and reproductive organs were weighed and eleven organs as well as gross lesions and tissue masses, if any, were collected and processed for histopathology. High dose and control groups were examined microscopically.</p> <p>NOAEL= 0.1g/kg , based on elevated liver weight and hematology effects at 0.5 g/kg (assigned by reviewer). No treatment related mortality or significant differences in food consumption or clinical signs, except for urogenital staining in high dose animals were observed. Body weight gain was significantly reduced in high dose males by 7% in wks 6-7 of dosing; wt gain in high dose females and all other treated animals was similar to controls. No apparent abnormalities in gross examination of visceral organs and no effects on organ weight of males in any group were observed. Absolute liver wt was elevated and brain wt was reduced in high dose females (p<0.05); brain wt was not significantly different from controls as a fraction of body weight. No treatment related microscopic changes in tissue from either sex in the high dose group were observed compared to controls. There were no gross lesions or masses. Erythrocyte counts, hemoglobin and hematocrit were significantly reduced in high dose females (p<0.01); hemoglobin was reduced in high dose males. Dose related changes in serum cholesterol (elevated) and SGOT levels (decreased) in high dose animals but fell within normal historical range of biological values. Other clinical chemistry parameters were not significantly different from concurrent vehicle control values.</p> <p>Administration of EDS coal-derived experimental fuel oil at doses up to 0.5g/kg/day for 13 weeks induced slight systemic toxicity in male and female rats, including reduced body wt gain, elevated liver weight, reduced hematology values, and elevated cholesterol which are qualitatively consistent with results of repeat exposure studies of other coal-derived liquids of similar boiling range.</p> <p>2. Reliable with restrictions. Analysis of dosing solutions</p>
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<p><u>References</u></p>	<p>was not performed for composition, purity or stability.</p> <p>McKee, R.H., Plutnick, R.T. and Traul, K.A. 1987. Assessment of the potential reproductive and subchronic toxicity of EDS coal liquids in Sprague Dawley rats. Toxicology 46: 267-289 (See separate summary for reproductive study).</p> <p>McKee, R.H. <i>et al.</i> J. Appl. Toxicol. 4: 198-205 (additional analytical data on fuel oil).</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Biphenyl Feedstock, CAS #68989-41-3. Dark brown liquid with crystal aggregates. Refer to sponsor for further information
<u>Method</u>	
Method/guideline followed	None specified. Comparable to standard study
Test type	Sub-acute
GLP	Yes
Year	1983
Species	Rat,
Strain	Fischer 344
Route of administration	Dermal
Duration of test	12 days
Doses/concentration levels	0, 1.0, 2.0g/kg (diluted in corn oil to constant volume of 4ml/kg for administration)
Sex	5M, 5F/dose group
Exposure period	6hr/day
Frequency of treatment	days 1-5, 8-11
Control group and treatment	5M, 5F; 4ml corn oil/kg
Post exposure observation period	None
Statistical methods	Mean and standard deviation, Bartlett's test, and analysis of variance. Dunnett's test if data were homogeneous; modified t-test if data were non-homogeneous.
<u>Test Conditions</u>	Rats were housed individually in suspended, stainless steel cages with mesh fronts and bottoms, equipped with an automatic watering system. Water and rat chow diet were available ad lib. Room temperature was maintained at 74 ⁰ F with a relative humidity of 55% and 12 hour light/dark cycle. Backs of rats were clipped of hair and dosed dermally on days 1-5 and days 8-11 at 0, 1.0, and 2.0g/kg diluted in corn oil. Elizabethan collars were applied for the 6 hour exposure period, after which collars were removed and skin wiped free of test article. Rats were sacrificed on day12. Rats were observed for mortality and moribundity twice daily on dosing days and once daily on non-dosing days. Body wt was recorded 5 days before initiation and on days 1, 8, and before sacrifice on day 12. Rats were observed once daily for clinical signs on dosing days. Dermal effects were scored twice on each dosing day - before application and after removal. Blood was taken for clinical chemistry and hematology 7 days prior to initiation

<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p> <p><u>Conclusions</u></p> <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>and on day 12 prior to sacrifice; parameters were total leukocyte count, total erythrocyte count, total platelets, hemoglobin, hematocrit, mean cell vol., mean cell hemoglobin, mean corpuscular hemoglobin, BUN, creatinine, alkaline phosphatase, sodium, potassium, glucose, SGOT, SGPT, total protein, albumin, and albumin/globulin ratio. All animals received a gross necropsy and potential target organs/tissues (liver, brain, heart, spleen, kidneys, testes) were weighed and preserved for histopathology. Certain organs/tissues (lung, skin, ovaries) were examined microscopically but not weighed. Only control and high dose groups were scheduled for histopatholgy.</p> <p>LOEL = 1g/kg based on reduction in body wt. (Assigned by reviewer)</p> <p>No mortality or moribundity was observed during the study. Body wts of males and females in groups 2 and 3 were reduced but the effect was statistically significant only in females. No dermal reactions or test article related clinical signs were observed. There were no biologically significant test article related changes in the hematology or clinical chemistry parameters. However, sera of test article-treated rats were more yellow than that of controls. There was an increase in absolute wt. of kidneys of males and females in the test article groups that was significant only for female left kidney. There were no gross pathological changes in skin at the site of application. There were no histopathological lesions that could be attributable to test article exposure.</p> <p>Dosing with the test article did not produce overt toxicological effects but there were decreases in terminal body wt and increases in specific organ wt that appeared to be treatment related</p> <p>2. Reliable with restrictions. Dosing solution concentrations were not verified by analysis.</p> <p>Rausina, G.A. 1983. Two-week repeated dose toxicity study in rats using Biphenyl Feedstock. Proj. #2044. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Biphenyl Feedstock, CAS #68989-41-3. Amber-colored, semi-solid with aromatic odor. Refer to sponsor for further information.
<u>Method</u>	
Method/guideline followed	None specified, comparable to standard study
Test type	Sub-acute
GLP	Yes
Year	1983
Species	Rat
Strain	Fischer 344
Route of administration	Inhalation
Duration of test	12 days
Doses/concentration levels	0, 1.07, 3.04g/m ³
Sex	5M, 5F/dose group
Exposure period	6 hr/day
Frequency of treatment	days 1-5, 8-11
Control group and treatment	5M, 5F; filtered air
Post exposure observation period	None
Statistical methods	Analysis of variance, Dunnett's test, Kolmogorov-Smirnov two-tailed test
<u>Test Conditions</u>	Rats were housed individually in stainless steel screen-bottomed cages in a room maintained at 75 ⁰ F with 37% relative humidity and 12 hour light/dark cycle. Chow diet and water were provided ad lib except during exposure. Test substance was aerosolized with a ball jet nebulizer heated to 70 ⁰ C to decrease viscosity. Chamber concentrations were determined by fluorescence spectroscopy. Both vapor phase and particulate phase concentrations were determined. Actual total exposure (and % of total as particulate) were 0, 1.07g/m ³ (60%) and 3.04g/m ³ (51%). Particulate mass median aerodynamic diameter was 5.2-5.4 microns. Rats were exposed to test article or filtered air for 6hr/day on days 1-5 and days 8-11; they were observed twice daily on dosing and once on non-dosing days for mortality and moribundity, and immediately after dosing for clinical signs. Body wt was recorded immediately prior to exposure on days 1 and 5, and prior to sacrifice on day 12. Non-fasted blood was collected on day 12 for blood chemistry and hematology. Measured chemistry parameters were BUN, creatinine, total protein,

<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p> <p><u>Conclusions</u></p> <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>albumin, albumin/globulin ratio, and SGPT. Hematology parameters were white blood cells, red blood cells (RBC), hemoglobin, hematocrit, mean RBC cell vol., mean RBC corpuscular vol., and mean corpuscular hemoglobin. All rats were necropsied for gross lesions. Organs weighed were liver, brain, heart, kidneys, spleen, testes, and lungs; these organs as well as ovary, uterus, eyes and nasal turbinates were saved for histopathological examination. Only organs/tissues from control and high dose rats were examined.</p> <p>LOEL = 1.07g/m³ based on body wt loss. Assigned by reviewer.</p> <p>There were no test article-related deaths during the study. Males and females of groups 2 and 3 showed dose related weight loss. Most of the group 2 and 3 rats showed perianal soiling, excessive ocular porphyrin, dry red matter around mouth and nose, and crystalline test article on fur. There were no biologically significant effects of test article on clinical chemistry or hematology parameters. However, there was a dose related yellowing of blood sera. There were significant increases in absolute liver and kidney wt of high dose females, and decreases in spleen wt of high dose males. There were many significant increases in organ/body wt ratios owing to decreased wt gain in treated rats. There were no microscopic changes in male or female organs/tissues attributable to test article exposure. The only effect noted at gross necropsy was skin discoloration in test article treated rats.</p> <p>Repeated exposure to 1.07g/m³ or 3.04g/m³ of test article caused no mortality. Several exposure-related clinical effects were observed including perianal soiling, respiratory and ocular discharges, and decreased body wt. In addition, at the high dose, female liver and kidney wts were increased and male spleen wt was decreased.</p> <p>1. Reliable without restrictions</p> <p>Gordon, T. 1983. Nine-day repeated dose inhalation study in rats, Biphenyl Feedstock. Proj. #2045. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Aromatic Pyrolysis Oil, CAS #64742-90-1. Black tarry material. Refer to sponsor for analysis of composition and purity.
<u>Method</u>	
Method/guideline followed	No guidelines specified, comparable to standard study.
Test type	Subacute
GLP	Yes
Year	1983
Species	Rat
Strain	Fischer 344
Route of administration	Dermal
Duration of test	2 weeks
Doses/concentration levels	0, 1, 2 g/kg
Sex	Males and females (5/sex/group), 7 wks old at initiation of study
Exposure period	6 hr./day for 9 days over a 14 day period
Frequency of treatment	once/day, 5 days/week
Control group and treatment	corn oil, 1.82 ml/kg
Post exposure observation period	None
Statistical methods	Bartlett's test, Analysis of Variance, Dunnett's test, Kolmogorov-Smirnov two-tailed test
<u>Test Conditions</u>	Animals were housed individually in suspended stainless steel cages with wire mesh bottoms and fronts in a room maintained at 69.8 ⁰ F with relative humidity of 46.5% and 12 hour light/dark cycle. Water and chow diet were provided ad lib. Doses of test article were administered over 10% of body surface to the backs of rats clipped free of hair and fitted with Elizabethan collars to reduce ingestion. After 6 hours, collars were removed and residual oil wiped off. Observations for mortality and morbidity were made twice/day on dosing days and once/day on non-dosing days, and for clinical signs at least once daily. Food consumption was determined weekly and body weight at study initiation and weekly thereafter. Dermal reactions were scored on each dosing day, before and after exposure. At sacrifice, gross necropsy was performed and liver, brain, spleen, heart, kidneys and testes were weighed. Slides of sections of the weighed organs, and also ovaries, uterus and skin were prepared for histopathologic examination of control and high dose groups.

<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p> <p><u>Conclusions</u></p> <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>NOEL not determined. LOEL = 1.0 g/kg No deaths or moribund rats were observed. Food consumption was decreased in all test article dosed rats; in males the decrease was dose related. In both males and females body weight was reduced in a dose related manner. Dermal effects were difficult to evaluate because of the black/tarry test material; however, in the high dose group, after a weekend without dosing, erythema was moderate to severe with fissuring and skin peeling. Skin histopathologic effects consisted of moderate to marked acanthosis and hyperplasia of the epithelium, and hyperkeratosis. No other test article related histopathologic lesions were found. There were no statistically significant changes in clinical chemistry and hematology parameters. Elevated absolute and relative liver weight was observed in all treated groups.</p> <p>Aromatic pyrolysis oil at 1.0 and 2.0 g/kg caused depression of body weight gain associated with decreased food consumption. At 2.0 g/kg, all rats showed moderate to severe erythema (Draize score 3-4). Fissures and peeling skin were seen at 2.0 g/kg but not at 1.0 g/kg. The 1.0 g/kg animals were not scored for erythema. Skin changes consisted of hyperplasia and acanthosis.</p> <p>1. Reliable without restrictions</p> <p>Zellers, J.E. 1983. Two week repeated dose toxicity study in rats using aromatic pyrolysis oil. Proj. #82-089. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX, USA. Unpublished report.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Aromatic Pyrolysis Oil, CAS #64742-90-1. Dark brown to black, highly viscous, tarry residue
<u>Method</u>	
Method/guideline followed	None specified; comparable to standard study.
Test type	Subacute
GLP	Yes
Year	1983
Species	Rat
Strain	Fischer 344
Route of administration	Whole body inhalation
Duration of test	12 days
Doses/concentration levels	0, 0.54, 2.00 g/m ³ (actual)
Sex	Male and female (5M, 5F/group)
Exposure period	9 days
Frequency of treatment	6 hr/day
Control group and treatment	filtered air, 6 hr/day for 9 days
Post exposure observation period	2 days
Statistical methods	Analysis of variance, Dunnett's test, Kolmogorov-Smirnov two tailed test.
<u>Test Conditions</u>	Animals (17 wks old) were housed individually and exposed to the test material for 6 hr/day in stainless steel dynamic exposure chambers. Temperature was maintained at 77 ⁰ F and relative humidity at 45%. Water and chow diet were provided ad lib. Nominal concentrations were measured gravimetrically and actual concentrations by fluorescence. Rats were observed twice daily on dosing days and once daily on non-dosing days for morbidity and mortality, and immediately after exposure for clinical signs. Body weight was taken at initiation, day 5 of treatment, and prior to sacrifice (day 12) after 2 days of recovery. Blood was collected via orbital sinus on day 12 for hematology and clinical chemistry. At sacrifice, necropsies were performed and target organs were weighed and preserved for histopathology.
<u>Results</u>	
NOAEL (NOEL)	NOEL not determined
LOAEL (LOEL)	LOEL = 0.54 g/m ³
Remarks	Males and females showed dose and time related decreases in body weight that were more severe in the male. Males

<p><u>Conclusions</u></p> <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>and females showed dose related increases in clinical symptoms (hair loss, nasal discharge, discharge from eyes, eyes closed and perianal soiling). At the high dose, one female showed arching of the back. Clinical pathology values were unremarkable. Treated male and female rats, showed yellow discoloration of the lungs grossly and hyperplasia of the pulmonary alveolar macrophages microscopically. The organ weights of the high dose males' and females' livers, the high dose females' lungs and the low dose females' livers were significantly increased relative to control animal values. The splenic weights of the high dose male and female rats were significantly decreased. A decrease was observed in the high dose male heart weights compared to control male data.</p> <p>No mortality or morbidity was observed. Exposure related signs included weight loss, hair loss, nasal and ocular discharge and arched walking. Skin irritation was observed at both dose levels, which resolved after the 2 days recovery period in the 0.54 g/kg dose group. There were several alterations in absolute and relative organ weights in both sexes at both test article doses. Frequency and severity of effects were related to exposure level.</p> <p>2. Reliable with restrictions. The report is not well written, key parameters that should have been noted in the methods section are scattered in the report. Most effects were clearly dose related but this was not evaluated statistically.</p> <p>Gordon, T. 1983. Nine-day repeated dose inhalation toxicity study in rats: Aromatic Pyrolysis Oil. Proj. #2035. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Rerun tower bottoms (RTB, 1271-81). Brown liquid. 140 ⁰ F (60 ⁰ C) vapor: 35% alkanes/alkenes, 60-65% benzene derivatives, and 2-3% naphthalenes. Benzene, toluene and styrene were 53% of vapor.
<u>Method</u>	
Method/guideline followed	Standard method, no guidelines specified.
Test type	Sub-acute toxicity from a Combined 10 day inhalation toxicity and teratology range-finding study
GLP	Yes
Year	1984
Species	Rat
Strain	Sprague Dawley [COBS:CD (SD)BR] from Charles River, UK
Route of administration	Whole body inhalation
Duration of test	12 days
Doses/concentration levels	0, 0.15, 0.74 and 5.1g/m ³ actual analyzed concentration
Sex	Males and females (10/sex/group)
Exposure period	10 consecutive days
Frequency of treatment	6 hr/day
Control group and treatment	10 males, 10 females exposed to room air, 6hr/day for 10 days
Post exposure observation period	None
Statistical methods	Bartlett's test for heterogeneity of variance. Body wt., food and water consumption employed analysis of variance (ANOVA). Hematology and blood chemistry used ANOVA, William's test. For organ wts, where Bartlett's test was significant at 1%, log transformation was used to see if heterogeneity was removed. Analysis of covariance or ANOVA followed by William's test was then used for significance.
<u>Test Conditions</u>	Male and female rats (approx 13 wks old; males 240-260g, non-pregnant females 180-200g) were housed 5/sex/cage in polypropylene cages with removable mesh tops and floors, on racks within individually ventilated areas between treatment periods to prevent passive exposure to RTB vapors from rats in other dose groups. Rooms were maintained at temperature range of 20-22 ⁰ C, relative humidity 60-64% and a 12 hr light/dark cycle. Rat chow and tap water were available ad lib except during exposure.

	<p>Rats were assigned to groups by body weight using a pseudo-random computer program. Exposure vapors were continuously generated from a supply of neat oil at 140⁰F in a double-surface glass condenser. Nitrogen gas carrier was passed through the condenser, diluted with air, and metered into the exposure chambers. Generation conditions at each dose level were designed to produce approximately 20% vaporization of RTB. Total chamber airflow was set for 12 air changes/hr. Chamber vapor concentrations were monitored by an infrared gas analyzer, and samples were collected every hour on carbon sorption tubes for analysis by gas chromatography/ flame ionization detection. Target concentrations were 0, 0.1, 0.75 and 5.0g/m³ (actual 0, 0.15, 0.74 and 5.1g/m³) determined from an acute inhalation study. During exposure, rats were housed in stainless steel mesh cages, partitioned to isolate each rat, set on supports in the inhalation chamber. Avg. chamber atmospheres were 23.5⁰F, 41-59% relative humidity. Positions of animals were alternated daily. After daily exposure was completed, chambers were allowed to clear for 30 min before the rats were removed and returned to holding cages. Animals were observed twice daily for clinical signs, before and after exposure as animals were moved between cages. Animals were weighed 7 days prior to exposure and on day 1 (time 0 of exposure), 3, 7, and 10, before daily exposure began. Daily food and water consumption was recorded by cage, beginning 7 days prior to start of exposure. Blood was drawn on day 11 (the day following the final exposure) from all animals (fasted 16hr overnight). Evaluated hematology parameters were hemoglobin, red cell (RBC) count, total white cell (WBC) count and differential WBC counts, and packed cell volume (PVC); blood chemistry parameters were plasma glucose, serum total protein, serum albumin, albumin/lobulin (A/G) ratio, urea nitrogen, alkaline phosphatase, and serum glutamic-pyruvic transaminase (GPT). On day 11, all rats were killed: lungs/trachea, liver, kidneys, spleen, thymus, adrenal and gonads were weighed; 34 tissues were preserved for possible microscopic examination. Detailed microscopic examination was performed on hematoxylin-eosin stained sections of liver, kidney, testes/ovary, trachea, lung and head from all rats; thymus, spleen and adrenal from control and 5.1g/m³ groups (10M, 10F) and spleen from 10 males only in the 0.74g/m³ group.</p>
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<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL) Remarks</p>	<p>NOAEL was not established LOAEL both sexes = 0.15g/m³ based on statistically significant dose-related changes in kidney wt and pathology for all males, decreased urea nitrogen levels in all females (Assigned by reviewer).</p> <p>No deaths occurred during the study. Clinical signs during exposure observed in the 5.1g/m³ group were abnormal body posture, closing or partial closing of eyes on every day of exposure consistent with exposure to an irritant atmosphere. Following exposure, some 5.1g/m³ animals exhibited lethargy, red/brown staining around head or snout, urine staining, salivation, occasional ataxia and lacrimation, peripheral vasodilation mainly on the first few days of exposure, and hair loss in a few rats at the end of the 10-day treatment period. Increased urination overnight was noted in these high dose rats (both sexes). Lower dose groups showed few clinical signs. Body wt and weight gain over 10 days were similar to controls for all animals in 0.15 and 0.74g/m³ groups (male gain: 39, 40, 40 g; female gain: 12, 13, 14g in control, 0.15 and 0.74g/m³ groups, respectively. At 5.1 g/m³, males failed to gain weight (-2g), resulting in a statistically significantly difference compared to controls; females gained less (8g) than controls but effect was not statistically significant. Overall, food consumption for 10 days was statistically significantly lower (p<0.01) in 5.1g/m³ males (202g) and females (149g) compared to controls (males, 248g, females, 168g), however female's food consumption became comparable to controls between days 6-9 of exposure. Other groups were similar to controls throughout. High dose rats of both sexes, particularly high dose females, consumed statistically significantly (p<0.01) more water than controls (males, 537g; females, 576g compared to control males, 324g; females 217g); all other groups were comparable to controls.</p> <p>Hematology parameters: Males exposed to 5.1g/m³ exhibited statistically significantly higher values for PCV, Hb, and RBC than controls, however the absolute values for individual animals were within historical control ranges for the laboratory; 5.1g/m³ females</p> <p>And other treated groups were comparable to controls.</p> <p>Blood chemistry parameters: the 5.1g/m³ males had significantly higher protein, albumin, and GPT levels and significantly lower alkaline phosphatase level than controls; in females the A/G ratio and GPT levels were higher than controls. The A/G ratio in 0.74g/m³ females was also</p>
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	<p>higher than controls. Urea nitrogen levels in females at all dose levels were statistically significantly lower than controls (21, 17, 19, and 14mg/dl in control, 0.15, 0.74 and 5.1g/m³, respectively). Again, absolute values for individual rats for these parameters were within acceptable ranges for age and strain of rat. At necropsy, increased incidence of alopecia and incidence of hemorrhagic areas in the mucosa of stomachs in 5.1g/m³ female rats was observed macroscopically. Liver, kidney and adrenal wt tended to be higher than controls for most groups in a dose-related fashion; spleen and thymus wt in 0.74g/m³ and 5.1g/m³ groups tended to be less than controls. Differences were statistically significant for males at all dose levels for kidney wt, at 0.74 and 5.1g/m³ for liver (increase) and spleen (decrease), and at 5.1g/m³ only for adrenal (increase) and thymus (decrease). In females, changes were statistically significant only at 5.1g/m³. Microscopic pathology results in the 5.1g/m³ group showed enlargement of centrilobular hepatocytes in liver (5/10 males, 8/10 females), decreased cellularity of red pulp (6/10males) in spleen, minimal involution of thymus (3/10 males, 3/10 females), minimal increase in fine vacuolation of zona fasciculata (6/10 males, 4/10 females), and minimal increase in cortical width (7/10 females, only) in adrenals. Male rats from all dose groups has eosinophilic intracytoplasmic inclusions in renal cortical tubules in the kidney. Other changes at 5.1g/m³ or in other dose groups were considered spontaneous in origin and of no toxicological significance.</p> <p><u>Conclusions</u></p> <p>NOAEL was not established. Decreases in body wt gain and food intake, increases in water intake, changes in hematology, blood chemistry and organ wts, and pathological findings were induced by rerun tower bottoms in rats exposed for 10 days. RTB appeared to induce diuretic action with compensatory water intake in both sexes. Incomplete compensation in male rats resulted in minimal hemoconcentration (increased PCV, Hb, RBC and total protein at 5.1g/m³); in female rats, a decrease in urea nitrogen (all doses) resulting from increased urine flow. Direct effect on the liver was demonstrated by elevated GPT levels, and enlargement of centrilobular hepatocytes in both sexes at the high dose. The occurrence of intracytoplasmic inclusions in renal cortical tubules was observed in male rats in all groups, increasing in severity with dose. [This effect could later be correlated with</p>
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<p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>characteristic hydrocarbon-induced nephropathy seen in male rats. Reviewer's comment]. Changes in adrenals, spleen and thymus probably correlate with stress response and adaptation to exposure to Rerun tower bottoms.</p> <p>1. Reliable without restrictions</p> <p>Rose, P.H., Street, A.E., James, P. and Gopinath, C. 1984. Compound 1271-81 vapor ten-day inhalation toxicity and teratology range-finding study in rats. HLS-MOB7/83656. Huntingdon Research Centre, plc. Cambridgeshire, England, for Mobil Oil Corp, Princeton, NJ, USA (see separate summary for teratology data).</p> <p>Rose, P.H., Jackson, G.C., Clark, G.C., and Gopinath, C. 1983. Acute inhalation toxicity in rats, 4-hour exposure. Report #MOB 9/83503. Huntingdon Research Center plc, Huntingdon, England, for Mobil Oil Corp., Princeton, NJ, USA.</p> <p>Roy, T.A. 1983. Analysis of rerun tower bottoms. Study #1271-81B,C. Mobil Oil Corp, Princeton, NJ, USA.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Pyrolysis Fuel Oil, (oil quenched). Tested as supplied by producer (Union Carbide). Produced by pyrolysis at temperatures up to 9500C, contained 300-500 ppm benzo(a)pyrene. Olefins Panel HPV Stream: Heavy Pyrolysis Fuel Oil (FO 2).
<u>Method</u>	
Method/guideline followed	No guidelines specified
Test type	Chronic/cancer study – skin painting
GLP	No
Year	1977 (in-life phase: May 1974 - September 1976)
Species	Mouse (40/group/dose)
Strain	C3H/HeJ
Route of administration	Dermal
Duration of test	Lifetime (28 months)
Doses/concentration levels	One brushfull – Mon, Wed., Fri.
Sex	Not specified
Exposure period	Continuous, no test article removal
Frequency of treatment	3 times/wk.
Control group and treatment	Distilled water and benzene concurrent controls - 1 brushfull 3 times/wk
Post exposure observation Period	None
Statistical methods	Least squares to determine median latent period
<u>Test Conditions</u>	Doses of neat test article or water or benzene were brushed on to the backs of mice, clipped free of hair, three times/wk. Doses were applied consistently to the midline but qualitatively, with each dose described as a brushfull. At monthly intervals, papilloma or carcinoma indexes were calculated (100 times the ratio of number of mice with skin tumors divided by the effective group). Effective group was calculated as original number of mice at start divided by the number dead without tumors, but maintained constant after appearance of the median tumor. Median papilloma or carcinoma latent periods were also determined. Lesions were verified by histopathology.
<u>Results</u>	
NOAEL (NOEL)	Not applicable
LOAEL (LOEL)	Not applicable
Remarks	Oil-quenched pyrolysis fuel oil was highly carcinogenic.

<p><u>Conclusions</u></p> <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>The papilloma and carcinoma indexes were 94.4 and 94.4, respectively, and the median papilloma and carcinoma latent periods were 10.3 and 12.1 months, respectively. The malignant tumors were squamous cell carcinomas. No tumors were observed in either control group.</p> <p>Pyrolysis fuel oil (oil quenched) was carcinogenic in the mouse skin painting bioassay.</p> <p>Reliable with restrictions. Although the method used the early qualitative procedure of actual skin painting rather than exact volume application, and the material was not analyzed, the results are unambiguous. The described process conditions and B(a)P levels are consistent with a dermal carcinogenic response and the latency period was short enough to anticipate possible metastatic spread.</p> <p>Weil, C.S., Condit, N.I. 1977. Experimental carcinogenesis of pyrolysis fuel oil. Am. Ind. Hyg. Assoc. J. 38: 730-733.</p>
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Repeated Dose Toxicity

<u>Test Substance</u>	Pyrolysis Fuel Oil, (water quenched). Tested as supplied by producer (Union Carbide). Produced by pyrolysis at temperatures up to 950 ⁰ C, contained 300-500 ppm benzo(a)pyrene.
<u>Method</u>	
Method/guideline followed	No guidelines specified
Test type	Chronic/cancer study – skin painting
GLP	No
Year	1977 (in-life phase: May 1974 - September 1976)
Species	Mouse (40/group/dose)
Strain	C3H/HeJ
Route of administration	Dermal
Duration of test	Lifetime (28 months)
Doses/concentration levels	One brushfull – Mon, Wed., Fri.
Sex	Not specified
Exposure period	Continuous, no test article removal
Frequency of treatment	3 times/wk.
Control group and treatment	Distilled water and benzene concurrent controls - 1 brushfull 3 times/wk
Post exposure observation period	None
Statistical methods	Least squares to determine median latent period
<u>Test Conditions</u>	Doses of neat test article or water or benzene were brushed on to the backs of mice, clipped free of hair, three times/wk. Doses were applied consistently to the midline but qualitatively, with each dose described as a brushfull. At monthly intervals, papilloma or carcinoma indexes were calculated (100 times the ratio of number of mice with skin tumors divided by the effective group). Effective group was calculated as original number of mice at start divided by the number dead without tumors, but maintained constant after appearance of the median tumor. Median papilloma or carcinoma latent periods were also determined. Lesions were verified by histopathology.
<u>Results</u>	
NOAEL (NOEL)	Not applicable
LOAEL (LOEL)	Not applicable
Remarks	Water-quenched pyrolysis fuel oil was highly carcinogenic. The papilloma and carcinoma indexes were 100 and 97.2,

<p><u>Conclusions</u></p> <p><u>Quality</u> Reliabilities</p> <p><u>References</u></p>	<p>respectively, and the median papilloma and cacinoma latent periods were 10.2 and 12.2 months, respectively. The malignant tumors were squamous call carcinomas. No tumors were observed in either control group.</p> <p>Pyrolysis fuel oil (water quenched) was carcinogenic in the mouse skin painting bioassay.</p> <p>Reliable with restrictions. Although the method used the early qualitative procedure of actual skin painting rather than exact volume application, and the material was not analyzed, the results are unambiguous. The described process conditions and B(a)P levels are consistent with a dermal carcinogenic response and the latency period was short enough to anticipate possible metastatic spread.</p> <p>Weil, C.S., Condia, N.I. 1977. Experimental carcinogenesis of pyrolysis fuel oil. Am. Ind. Hyg. Assoc. J. 38: 730-733.</p>
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Toxicity to Reproduction

<u>Test Substance</u>	EDS Experimental fuel oil: 70/30 (w/w) blend of recycle solvent and vacuum gas oil. Produced by EDS Coal Liquefaction Plant process run on Illinois No. 6 coal: 62.7% aromatics, 37.3% saturates (13.9% paraffins, 23.4% naphthenes). Nominal boiling range: 200-538 ⁰ C.
<u>Method</u>	
Method/guideline followed	Other
Test type	1-generation reproductive toxicity
GLP	Yes
Year	1985 (1987 publication)
Species	Rat
Strain	Sprague-Dawley (Hilltop Laboratory Animals, Scottdale, PA, 6 wks old at receipt)
Route of administration	Oral gavage
Duration of test	Approx. 142 days (90 days exposure, 10 days mating, 40-42 days gestation and lactation)
Concentration levels	0, 0.02, 0.1, 0.5 g/kg/day diluted in highly refined white oil (CAS #8012-95-1)
Sex	Male and female (18M, 36F/dose group).
Exposure period	13 weeks (90 days)
Frequency of treatment	once a day, 5 days/wk
Control group and treatment	36M, 72F; 5ml/kg white oil; once a day, 5 days/wk
Statistical methods	Bartlett's test of homogeneity of variance, standard ANOVA, Duncan's test, linear regression for dose response; Kruskal-Wallis (non-parametric test) followed by Dunn's Summed Rank test if appropriate; Jonckheeres test for monotonic trends in dose groups. Standard nested ANOVA for fetal body parameters by sex nested within dam and dam within dose group analysis followed by least significant differences technique.
<u>Test Conditions</u>	This assay was the reproductive portion of a combined Reproductive/Subchronic toxicity study with an initial group size of 18M, 54F/dose group and 36M, 90F/control. Animals were assigned to 4 treatment groups based on body weight and were housed individually in stainless steel cages in rooms maintained at 20-24 ⁰ C temperature, 40-70% relative humidity and 12 hr light/dark cycle. Animals received water and food ad lib. Test material was administered by gavage 5 times weekly for 13 wks; body weight was recorded prior to dosage initiation (day 0) and

<p><u>Results</u> NOAEL Remarks</p>	<p>weekly throughout the exposure period. At the end of 13 weeks of treatment, 18 female rats/group were selected for the subchronic study and the others mated. All males were mated, then killed and necropsied for the subchronic study. 18M, 36F/dose group; 36M, 72F/control group were housed together (1M:2F) for 10 consecutive nights or until mating was confirmed by vaginal plug or sperm in a vaginal rinse = Gestation Day (GD) 0. Mated females were maintained untreated throughout gestation and lactation (LD) to postpartum day 21. Maternal body weight was recorded on GD 0, 7, 14, 21, and LD 0, 7, 14, 21 and all dams were examined for viability, toxicological effects and unusual behavior. Offspring were examined grossly and weighed at LD 0, 4, 14, 21. All dams and surviving litters were killed and grossly examined on LD21. Pups were necropsied and visceral organs and brain examined. Pups that died spontaneously were also necropsied unless precluded by autolysis or cannibalism.</p> <p>NOAEL (maternal)= 0.5g/kg; NOAEL (offspring)=0.5g/kg (assigned by reviewer).</p> <p>No treatment related mortality or significant differences in body weight gain, food consumption or physical signs, except for urogenital staining in high dose animals were observed. Seven control animals, 1 low dose female, and 1 mid dose male were euthanized during the treatment period due to dosing injuries. Pregnancy indices were comparable in all dose groups: 80.9%, 86.1%, 88.9% and 83.3% in control, low, mid and high dose groups respectively. No significant differences were observed in length of gestation or in maternal weight gain during gestation. No treatment related effects were observed in mean litter size, live births or pup survival at LD 4, 14 and 21 or in pup body weight throughout lactation to weaning at LD21. Sex of pups was not reported. A low incidence of malformations occurred in all groups: Control: 4 pups in 4 litters – 2 tail abnormalities, 1 depressed sternum, 1 short snout. Low dose: 4 pups in 2 litters – 2 syndactyly, renal agenesis, no sex organs (1 agnatha), 1 syndactyly and renal agenesis, 1 pup with small kidney. Mid dose: 2 pups in 2 litters – tail abnormalities. High dose: 4 pups in 2 litters – 1 agnatha, 2 tail abnormalities, 1 misshapen skull.</p> <p>Syndactyly and renal agenesis have been reported as a spontaneously occurring genetic syndrome in Sprague-</p>
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<p><u>Conclusions</u></p> <p><u>Data Quality</u> Reliability</p> <p><u>Reference</u></p>	<p>observations in these groups that included nasal, ocular, oral, and vaginal discharges, rales, and ungroomed appearance.</p> <p><u>Fetal:</u> The mid and high dose groups exhibited significant increases in early embryonic resorptions with corresponding decreases in the mean number of live fetuses (86% and 25% compared to 100% in the control and low dose). The remaining fetuses in the mid and high dose group had significantly reduced fetal body weight and crown-rump distance. The overall number of fetal skeletal malformations were not significantly different from the controls, although the ratios of malformed fetuses per litter were significantly increased in the mid and high groups. Gross visceral abnormalities were observed only in the mid and high dose groups.</p> <p>Coal-derived experimental fuel oil administered orally to pregnant female rats was embryo-lethal and teratogenic in rats at doses that are maternally toxic. Under the conditions of this study, the test substance was not a selective developmental toxicant.</p> <p>2. Reliable with restrictions. Analysis of dosing solution for composition, purity or stability. was not performed</p> <p>Exxon Biomedical Sciences (1984). Oral teratology study in rats. Unpublished report.</p>
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Developmental Toxicity/Teratogenicity

<u>Test Substance</u>	Rerun tower bottoms (RTB, 1271-81). Brown liquid. 140 ⁰ F (60 ⁰ C) vapor: 35% alkanes/alkenes, 60-65% benzene derivatives, and 2-3% naphthalenes. Benzene, toluene and styrene were 53% of vapor.
<u>Method</u>	Standard method, no guidelines specified.
Method/guideline followed	Teratology range-finding from a Combined 10 day inhalation toxicity and teratology range-finding study
Test type	
GLP	Yes
Year	1984
Species	Rat
Strain	Sprague Dawley [COBS:CD (SD)BR] from Charles River, UK
Route of administration	Whole body inhalation
Concentration levels	0, 0.15, 0.74 and 5.1g/m ³ actual analyzed concentration
Sex	Time-mated females (5/ group)
Exposure period	Day 6-15 of gestation
Frequency of treatment	6 hr/day
Control group and treatment	5 time mated females, exposed to room air 6hr/day from day 6-15 of gestation
Duration of test	20 days
Statistical methods	None performed. Small sample size in range-finding study
<u>Test Conditions</u>	Twenty sexually mature time mated female rats (171-200g at study initiation) were assigned to one of four groups (5/group) based on body wt on day 1 of gestation. The day of mating identified by presence of a vaginal plug =day 0 of gestation. Rats were housed 5/group in polypropylene cages with stainless steel mesh floors and tops when not in exposure chambers. Animal rooms were maintained at 20-22 ⁰ C temperature, 60-64% relative humidity and a 12hr light/dark cycle. Rat chow and tap water were available ad lib except during exposure. Exposure vapors were continuously generated from a supply of neat oil at 140 ⁰ F in a double-surface glass condenser. Nitrogen gas carrier was passed through the condenser, diluted with air, and metered into the exposure chambers. Generation conditions at each dose level were designed to produce approximately 20% vaporization of RTB. Total chamber airflow was set for 12-air changes/hr. Chamber vapor concentrations were monitored by an infrared gas analyzer, and samples were

<p><u>Results</u> NOAEL maternal toxicity NOAEL developmental toxicity Maternal effects</p>	<p>collected every hour on carbon sorption tubes for analysis by gas chromatography/ flame ionization detection. Target concentrations were 0, 0.1, 0.75 and 5.0g/m³ (actual 0, 0.15, 0.74 and 5.1g/m³) determined from an acute inhalation study. During exposure, rats were housed in stainless steel mesh cages, partitioned to isolate each rat, set on supports in the inhalation chamber. Avg. chamber atmospheres were 23.5⁰F, 41-59% relative humidity. Positions of animals were alternated daily. After daily exposure was completed, chambers were allowed to clear for 30 min before the rats were removed and returned to holding cages. Animals were observed twice daily for clinical signs, before and after exposure, and from post-treatment days 16-20 of gestation. Rats were weighed on day 1, 3, 6, 10, 14, 17 and 20 of gestation. Food and water consumption were recorded daily by cage. On day 20 of gestation, rats were killed by CO₂ asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. Ovaries and uteri were examined for number of corpora lutea, number and distribution of live fetuses, embryonic/fetal death, fetal abnormalities and individual live fetal wt from which litter wt was calculated. Uteri or individual uterine horns without visible implantations were immersed in 10% ammonium sulfide to evaluate pregnancy. Live fetuses were examined externally and weighed. One half of fetuses in each litter were preserved in Bouin's fixative for soft tissue evaluation by free-hand sectioning if required, one half were fixed, sexed internally, eviscerated, cleared and stained with Alizarin Red for skeletal examination if required. Group mean values for litter size, embryonic death, and pre- and post-implantation loss were calculated.</p> <p>NOAELmaternal = 0.74g/m³ (Assigned by reviewer) NOAEL developmental = 0.15g/ m³ (Assigned by reviewer)</p> <p>During exposure, 5.1g/m³ females only showed closing or partial closing of eyes, inactivity and abnormal body posture. Between exposures, 5.1g/m³ females showed lethargy, red staining of snout, slight general vasodilation, and increased urination and staining of urogenital region with occasional signs of slight ataxia and increased salivation. At 0.74g/m³, one rat had red staining on snout on 2 occasions. At 5.1g/m³, food consumption was reduced during the treatment period and water consumption was</p>
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<p>Embryo/fetal effects</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>References</u></p>	<p>markedly increased during and post-treatment; other groups were similar to controls. At 5.1g/m³, body wt loss occurred during the first four days of treatment (-1.0g compared to 21.2, 25.0 and 19.3g in control 0.15, and 0.74g/m³ groups, respectively); body wt gain in the 0.74g/m³ group was marginally lower than controls, but regained parity by day 14. From days 14-20 of gestation, high dose weight gain improved but did not reach parity with other dose groups. At necropsy, no gross abnormalities were observed in parental animals. Pregnancy rate was 80-100% in all groups, incidence of corpora lutea, implantations, and live young were comparable to or higher in treated groups than controls.</p> <p>At 5.1g/m³, mean fetal wt was markedly reduced (2.97g compared to 3.57g in controls) and mean number of intra-uterine deaths was higher (1.6 compared to 0.6 in controls); these effects of lesser magnitude were also observed in the 0.74g/m³ group litters. No fetal malformations were observed at necropsy.</p> <p>Rerun tower bottoms induced clinical signs of exposure in pregnant rats, principally at the highest dose level, 5.1g/m³, and marginal body wt loss and decreased food consumption during the early days of exposure; increased water consumption was observed throughout exposure and the post-treatment period. Mean fetal wt was reduced and mean number of intra-uterine deaths was increased at 5.1g/m³. No teratogenic effects (malformations or variations) were observed in offspring at necropsy. The doses employed in this assay would be acceptable for a definitive teratology study of Rerun tower bottoms.</p> <p>1. Reliable without restrictions</p> <p>Rose, P.H., Street, A.E., James, P. and Gopinath, C. 1984. Compound 1271-81 vapor ten-day inhalation toxicity and teratology range-finding study in rats. HLS-MOB7/83656. Huntingdon Research Centre, plc. Cambridgeshire, England, for Mobil Oil Corp, Princeton, NJ, USA (see separate summary for toxicity data).</p> <p>Rose, P.H., Jackson, G.C., Clark, G.C., and Gopinath, C. 1983. Acute inhalation toxicity in rats, 4-hour exposure. Report #MOB 9/83503. Huntingdon Research Center plc, Huntingdon, England, for Mobil Oil Corp., Princeton, NJ,</p>
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	<p>USA.</p> <p>Roy, T.A. 1983. Analysis of rerun tower bottoms. Study #1271-81B,C. Mobil Oil Corp, Princeton, NJ, USA.</p>
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Developmental Toxicity/Teratogenicity

<u>Test Substance</u>	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
<u>Method</u> Method/guideline followed Test type GLP Year Species Strain Route of administration Concentration levels Sex Exposure period Frequency of treatment Control group and treatment Duration of test Statistical methods	Standard method, no guidelines specified. Teratology study Yes 1981 Rabbit New Zealand White Dermal 0, 62.5, 125, 250, 500 and 1000mg/kg/day Females (5/ group) Day 6-18 of gestation Once daily 5 females/group, distilled water applied dermally from day 6-18of gestation 29 days None performed. Small sample size in range-finding study
<u>Test Conditions</u>	Thirty sexually mature, virgin female rabbits (8.5-9.5 months old at time of insemination), had been acclimated for 30 days, ear-tagged with a unique number for identification, and assigned by body wt to one of 5 groups (5 females/group). All rabbits were individually housed in suspended wire mesh cages and maintained in a temperature and humidity controlled room (specifics not given) with a 12hr light/dark cycle. Rabbit chow and tap water was available ad lib. During the treatment period, all animals were maintained in a specially ventilated room under identical housing conditions. Females were artificially inseminated with semen from one of 3 proven male rabbits of same strain and source. Semen was collected using an artificial vagina; only semen with 50% or greater motility was used. Ejaculate was diluted with 4.0ml of 0.9% NaCl, USP injectable grade at 35°C and 0.25-0.5ml was introduced into the anterior vagina. Within one hour after insemination, ovulation was induced by injection of 100units of chorionic gonadotropin into the marginal ear vein. Day of insemination =day 0 of gestation. Semen

<p><u>Results</u> NOAEL maternal toxicity NOAEL developmental toxicity Maternal effects</p>	<p>from one male was used to inseminate an equal number of females in each group. Prior to test article administration (gestation day 6) and as often as necessary thereafter, the back of each female was shaved and each animal was fitted with a Saf-T Shield® collar. Rerun tower bottoms was applied undiluted to the intrascapular area in a single daily application on gestations days 6-18. The site was uncovered, non-abraded and was not washed. Control rabbits were treated with distilled water at a concentration of 1.08ml/kg in a comparable regimen. Dosages were determined from individual body wt at day 6; collars were removed on gestations day 19. Prior to, during treatment, and until day 29 of gestation, females were observed for mortality, overt changes in appearance and behavior, and clinical signs of toxicity (day 6-29 of gestation). Females showing signs of abortion remained on study until scheduled sacrifice to determine any test article related effect; aborted tissue was discarded. Gross necropsy was performed on all rabbits not surviving to scheduled necropsy. Maternal body wt were recorded on gestation days 0, 6, 12, 18, 24 and 29. Dams were sacrificed on day 29, uteri were excised and the location of viable and non-viable fetuses, early and late resorptions, number of total implantations and corpora lutea were recorded. Abdominal and thoracic cavities and organs of dams were examined for gross evidence of morphological changes and discarded. Uteri from apparent non-gravid females were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy status.</p> <p>NOAEL maternal was not established (Rabbits at lowest dose aborted) NOAEL developmental was not established. (Reviewer's comments).</p> <p>All animals in control, 62.5, 125 and 250mg/kg groups survived to the end of the study. One 500mg/kg female aborted and died on gestation day 229, and 3 died in the 1000mg/kg day group – one on day 21, 2 on day 27 of gestation (1 aborted prior to death). Prior to death, matting and/or staining of hair in the urogenital region and lack of coordination of the hind limbs was noted. At necropsy, reddened foci and/or focal erosion of stomach lining was observed, suggesting that, despite collars, inherent preening activity and subsequent oral ingestion of RTB occurred. Two females that died in the</p>
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<p>Embryo/fetal effects</p> <p><u>Conclusions</u></p>	<p>1000mg/kg group had tan, creamy intestinal contents; distention of gall bladder was also noted in one of these rabbits. In the 1000mg/kg rabbit that died on day 21 of gestation, the left uterine horn was 1/3 as long as the right horn with thickening of the endometrium. Eleven rabbits aborted: 2 each in control, 62.5 and 500mg/kg/day groups, 3 in 125mg/kg group and 1 each in the 250 and 1000mg/kg groups. Since 40% (2/5) control rabbits aborted, inhalation of RTB may be a contributing factor. Dermal irritation at application site, erythema, edema, fissuring, was observed in all treated rabbits, first reported on day 13 of gestation and persisting until sacrifice. Eschar formation was reported later in the treatment period (days not given) and persisted until sacrifice. Nasal discharge, soft stool, occasional instances of dry, yellow matter in nasal region, hair loss, and white ocular discharge were noted in controls and treated animals with similar frequency. At necropsy, one female in the 62.5mg/kg group had a 2cm diameter abscess on the ventral neck; one 125mg/kg female had several erosions in the lining of the gall bladder, and one female had slight pitting of kidneys; one 500mg/kg female had accentuated lobulation of the liver. Fluid in the abdominal cavity was observed in one 62.5mg/kg female and one 500mg/kg female. Mean maternal body wt losses occurred in all treated groups during the treatment period (day 6-18 of gestation). Mean body wt gains were seen following the end of exposure (day 18-29 of gestation) in the 62.5, 125 and 500mg/kg groups; mean gains exceeded controls. Continued wt loss occurred in the 250 and 1000mg/kg groups post-treatment. Post-implantation loss occurred in the control group and in treated groups below or within laboratory historical control values (0.4-1.6); losses did not occur in a dose-related pattern. No biologically meaningful differences were observed in mean number of corpora lutea, total implantations or viable fetuses in any treatment group compared to controls. Decrease in mean number of total implants correspond to decrease in mean number of viable fetuses in 125 (one dam), 250 and 500mg/kg/day groups- one dam in each group had only early resorptions. Mean number of total implants and viable fetuses in 1000mg/kg group was greater than control mean values (9.5 implants and 8.5 fetuses in 1000mg/kg group compared to 6.7 implants and 6.7 fetuses in controls). No grossly observed teratogenic effects were reported.</p> <p>Dermal irritation, erythema, edema, fissuring and eschar</p>
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<p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>References</u></p>	<p>formation, were induced by Rerun tower bottoms in all treated groups. Mean maternal body wt losses occurred during treatment (day 6-18 of gestation). Four rabbits died (1 in 500mg/kg, 4 in 1000mg/kg group); 2 of which aborted prior to death. Eleven rabbits aborted, including 2 in control group, suggesting that inhalation of RTB may have been a contributing factor. Due to high abortion rate in every treatment group, a dose of 62.5mg/kg/day would be excessive for definitive teratology study.</p> <p>1. Reliable without restrictions</p> <p>Spicer, E.J.F and Schardein, J.L. 1981. Pilot dermal teratology study in rabbits (MCTR-169-79). IRDC Proj. #450-013. International Research and Development Corp., Mattawan, Mich. for Mobil Oil Corp., Princeton, NJ, USA.</p> <p>Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ, USA.</p>
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Developmental Toxicity/Teratogenicity

<u>Test Substance</u>	Heavy pyrolysis hydrocarbons (Rerun Tower Bottoms). Liquid, 40-50% C5-C8 aliphatic hydrocarbons and 40-50% aromatic hydrocarbons ranging from alkyl benzenes to alkyl naphthalenes. Analysis: 0.98% C5, 0.2% C6, 0% C7, 4.9% C8, 4.4% C9, 89.2% C10+
<u>Method</u> Method/guideline followed Test type GLP Year Species Strain Route of administration Concentration levels Sex Exposure period Frequency of treatment Control group and treatment Duration of test Statistical methods	Standard method, no guidelines specified. Teratology study Yes 1981 Rabbit New Zealand White Dermal 0, 10, 25, and 50mg/kg/day Females (16/ group) Day 6-18 of gestation Once daily Proximate and remote control groups: 16 females/group, distilled water applied dermally from day 6-18of gestation 29 days All analyses compared proximate to remote control and each control group to each treatment group (p<0.05). Chi square with Yates correction and/or Fisher's exact probability test used for male to female sex distribution and number of litters with malformations. Mann-Whitney U-test used for number of early and late resorptions and post-implantation losses. One-way analysis of variance, Bartlett's test, appropriate t-test for equal or unequal variance and Dunnett's multiple comparison tables were used for other fetal parameters and mean fetal body wt.
<u>Test Conditions</u>	Eighty sexually mature, virgin female rabbits (approx. 7 months old at time of insemination), had been acclimated for 63-68 days, ear-tagged with a unique number for identification, and assigned by body wt to one of 3 groups (16 females/group). All rabbits were individually housed in suspended wire mesh cages and maintained in a temperature and humidity controlled room (specifics not given) with a 12hr light/dark cycle. Rabbit chow and tap water was available ad lib. During the treatment period, all animals were maintained in a specially ventilated room under identical housing conditions. Remote control females were

	<p>housed separately. Females were artificially inseminated with semen from one of 10 proven male rabbits of same strain and source. Semen was collected using an artificial vagina; only semen with 50% or greater motility was used. Ejaculate was diluted with 4.0ml of 0.9% NaCl, USP injectable grade at 35°C and 0.25-0.5ml was introduced into the anterior vagina. Within one hour after insemination, ovulation was induced by injection of 100units of chorionic gonadotropin into the marginal ear vein. Day of insemination =day 0 of gestation. Procedures were performed on 2 consecutive days and equal numbers of females were inseminated in each group/day. Prior to test article administration (gestation day 6) and as often as necessary thereafter, the back of each female was shaved and each animal was fitted with a Saf-T Shield® collar. Doses were selected following a pilot range-finding study. Rerun tower bottoms was applied undiluted to the intrascapular area in a single daily application on gestations days 6-18. The site was uncovered, non-abraded and was not washed. Control rabbits were treated with distilled water in a comparable regimen. Dosages were determined from individual body wt at day 6; collars were removed on gestations day 19-20.</p> <p>Prior to, during treatment, and until day 29 of gestation, females were observed for mortality, overt changes in appearance and behavior, and clinical signs of toxicity (day 6-29 of gestation). Females showing signs of abortion were sacrificed and examined for grossly evident morphological changes; intact fetuses were examined externally and preserved in formalin. Gross necropsy was performed on all rabbits not surviving to scheduled necropsy, and tissue was saved in formalin, if appropriate. Maternal body wt were recorded on gestation days 0, 6, 12, 18, 24 and 29. Due to a weighing error on gestation day 18, body wt from 8 females in the 25mg/kg group were not included in calculations of group mean. Dams were sacrificed on day 29, uteri were excised and weighed prior to removal of fetuses. The location of viable and non-viable fetuses, early and late resorptions, number of total implantations and corpora lutea were recorded. Abdominal and thoracic cavities and organs of dams were examined for gross evidence of morphological changes and discarded. Uteri from apparent non-gravid females were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy status. All fetuses were individually weighed and examined for external malformations and variations.</p>
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<p><u>Results</u> NOAEL maternal toxicity NOAEL developmental toxicity Maternal effects</p>	<p>Each was internally sexed and examined for visceral malformations and variations including brain by mid-coronal slice, and heart dissection by modification of Staples' technique. Eviscerated skinned fetuses were individually numbered and tagged, fixed in alcohol, macerated in KOH and stained with Alizarin red S for skeletal examination.</p> <p>NOAEL maternal was not established (Dermal irritation at all levels) NOAEL developmental = 50mg/kg/day (Levels assigned by reviewer).</p> <p>All animals in control, 10, and 25mg/kg groups survived to the end of the study. One 50mg/kg non-gravid female died on gestation day 11, exhibiting edematous lungs and hyperemic tracheal mucosa at necropsy, suggesting respiratory distress as the cause of death. There were 14/16 apparently gravid rabbits in the proximate control, 10/16 in the remote control, 14/16, 12/16 and 14/16 in the 10, 25 and 50mg/kg groups, respectively. Eight rabbits aborted and were sacrificed; one each in proximate control (gestation day 23) and 25 mg/kg group (gestation day 26), 2 in 10mg/kg group (gestation day 26), and 4 in 50mg/kg group (One each on gestation day 20, 22,27, 28). Majority of rabbits that aborted in the 50mg/kg group had congested lungs or foci on lungs at necropsy. Dermal irritation at application site was observed in all treatment groups. Redness and swelling of application site was evident at initiation of treatment and persisted throughout gestation. Majority of females developed fissuring and flaking midway through treatment that persisted until sacrifice. Peeling of epidermal layer was reported in 7 rabbits, one at 10, 2 at 25 and 4 at 50mg/kg groups for several days during the treatment period. Dermal irritation did not occur in either control group. Soft stool was evident in the majority of rabbits treated with 50mg/kg/day. No treatment related findings were observed in organs of maternal females at necropsy. There were no biologically meaningful differences in mean maternal body wt gain between controls, or between any treated group and remote controls during the treatment period (day 6-18 of gestation)- gains were 147,212, 145 and 148 in remote control, 10, 25 and 50mg/kg/day groups, respectively. Comparable losses in mean adjusted body wt (female body wt on gestation day 29 minus uterus and contents) were observed in both controls</p>
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<p>Embryo/fetal effects</p> <p><u>Conclusions</u></p>	<p>and all treated groups [-104, -272, -55, -171, -244 in proximate controls, remote controls, 10, 25 and 50 mg/kg/day, respectively. Since a substantial adjusted body wt loss was observed in remote control animals, these changes were not considered treatment- related.</p> <p>At caesarean section, there were no statistically significant or biologically meaningful differences in mean number of corpora lutea, total implantations, post-implantation loss, early or late resorptions, viable fetuses or fetal sex distribution between controls or in 10, 25 or 50mg/kg/day groups. The incidence of gravid dams with viable fetuses was 100% in all groups: 13, 10, 12, 11, and 10 and viable fetuses/dam were 6.7, 6.7, 5.6, 7.1, and 7.0 in proximate control, remote control, 10, 25, and 50mg/kg/day groups. A decrease in mean number of corpora lutea. A decrease in mean number of corpora lutea and total implantation with corresponding decrease in mean number of viable fetuses was observed in the 10mg/kg day group when compared to remote controls, but effect were not observed at higher doses and were not considered treatment related. A lower mean fetal body wt. was observed at the 50mg/kg/day level (36.4g compared to 40.4g in proximate control and 42.9g in remote controls) but was within the laboratory's historic control range (35.2-42.6g). There were no biologically meaningful or statistically significant differences in number of litters with malformations in either control group or RTB-treated groups. Hydrocephaly was observed in 4 fetuses in one litter in the 10mg/kg group and 3 fetuses in 2 litters (2 in one litter and 1 in one litter) in the 25mg/kg/day group. No hydrocephaly was seen at 50mg/kg/day so these finding were considered not treatment related. Spina bifida occurred in one fetus in one 50mg/kg/day group litter. An increase in number of fetuses and number of litters with 13th rudimentary rib(s) occurred in the 50 mg/kg/day group which slightly exceeded the historical control range; % incidence was 25.7% affected fetuses in 80.5% litters compared to 21.8% affected fetuses in 61.5% litters in proximate control and 23.9 affected fetuses in 70% litters in remote control groups. All other genetic and developmental variations were within historical control range.</p> <p>Dermal irritation was observed in all rabbits treated with Rerun tower bottoms with a dose-related increase in number of females exhibiting peeling of the epidermal layer. One 50mg/kg/day non-gravid female died during gestation, probably the result of respiratory distress. Eight rabbits</p>
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<p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>References</u></p>	<p>aborted, one each in proximate controls and 25mg/kg group, 2 in 10mg/kg group, 4 in 50 mg/kg group; no abortions occurred in remote control animals. The reason for the high abortion rate maybe consequence of severe dermal irritation in treated groups and inhalation of RTB vapor was a contributing factor. No significant differences in mean maternal or fetal observations at caesarean section or in number of litters with malformations and genetic or developmental variations were identified in RTB-treated groups compared to controls. No fetal toxicity was observed and Rerun tower bottoms did not produce a teratogenic response at any treatment level.</p> <p>1. Reliable without restrictions</p> <p>Spicer, E.J.F and Schardein, J.L. 1981. Dermal teratology study in rabbits (MCTR-169-79). IRDC Proj. #450-009. International Research and Development Corp., Mattawan, Mich. for Mobil Oil Corp., Princeton, NJ, USA.</p> <p>Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81. Toxicology division, Mobil Oil Co., Princeton, NJ, USA.</p>
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AQUATIC TOXICITY ROBUST SUMMARIES**Fish Acute Toxicity**

Test Substance:	<p>Industry Stream Name: Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation)</p> <table> <tr> <td><u>CAS Number</u></td> <td><u>CAS Inventory Name</u></td> </tr> <tr> <td>68513-69-9</td> <td>Residues, petroleum, steam-cracked light</td> </tr> <tr> <td>68921-67-5</td> <td>Hydrocarbons, ethylene-manuf.-by-product distn.</td> </tr> <tr> <td></td> <td>residues</td> </tr> </table> <p>This stream is separated by distillation from pyrolysis gasoline, as a bottoms product. The composition indicates a carbon number distribution from C9 or C10 to hydrocarbons boiling at 650°F or higher. The reported typical composition includes 20% dicyclopentadiene, 30% codimers of C5 and C6 monomers, 20% naphthalene and substituted naphthalenes.</p>	<u>CAS Number</u>	<u>CAS Inventory Name</u>	68513-69-9	Residues, petroleum, steam-cracked light	68921-67-5	Hydrocarbons, ethylene-manuf.-by-product distn.		residues
<u>CAS Number</u>	<u>CAS Inventory Name</u>								
68513-69-9	Residues, petroleum, steam-cracked light								
68921-67-5	Hydrocarbons, ethylene-manuf.-by-product distn.								
	residues								
Method/Guideline:	OECD Guideline 203								
Year (guideline):	1992								
Type (test type):	Fish Acute Toxicity Test								
GLP (Y/N):	Yes								
Year (study performed):	2003								
Species:	<i>Oncorhynchus mykiss</i>								
Analytical Monitoring:	Yes								
Exposure Period:	96 hours								
Statistical Method:	<p>The 6-hour and 24-hour LL₅₀ and LC₅₀ values were determined using a maximum likelihood analysis based on D. J. Finney, 1971. A Trimmed Spearman-Kärber Method (Hamilton <i>et al.</i>, 1977) was used to determine the 48-hour, 72-hour and 96-hour LL₅₀ and LC₅₀ values.</p> <p>Finney, D.J., 1971. Probit Analysis, 3rd Edition, London: Cambridge University Press.</p> <p>Hamilton, M., R. Russo, R. Thurston, 1977. Trimmed Spearman-Kärber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. <i>Environmental Science and Technology</i>, Vol. 11, No. 7, p.714-719.</p>								

<p>Test Conditions:</p> <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, volume, replication, environmental conditions, organisms supplier, loading, deviations from guideline or protocol. 	<p>Individual Water Accommodated Fractions (WAF's) were prepared for each treatment. The test substance was added to 18 L of reconstituted water in glass aspirator bottles (capacity 22 L). The solutions were mixed for 24 hours using a 3% vortex (of the static liquid depth). The test solutions were removed through the outlet at the bottom of each mixing vessel into three replicates of 4.5 L in 4 L size aspirator bottles (no headspace). Four fish were added to each replicate and the replicates were closed with foil covered neoprene stoppers. Daily renewals were performed by removing ~90% of the test solution through the outlet at the bottom of the aspirator bottle and refilling with fresh solution. The fish were received from Thomas Fish Company, Anderson, CA. The fish were not fed during the study. They were held for 13 days in study dilution water prior to use and were 29 days old at the start of the study. Fish mean weight = 0.206 g, mean total length = 3.1 cm, test loading = 0.183 g of fish/L.</p> <p>Mean test temperature: 13.6°C (S.D. = 0.1), diurnal light: approximately 16 hours light and 8 hours dark with 607 to 614 Lux during full daylight periods. Dissolved oxygen ranged from 6.8 to 8.6 mg/L and pH ranged from 7.3 to 8.1 during the study. Water hardness was 104 mg/L as CaCO₃.</p> <p>Due to the complex nature and limited water solubility of the test substance, the following exceptions to the guideline apply for this study: The concentration of the test substance in solution was not determined prior to use. The initial concentration of the test substance was not maintained at 80% in the highest loading rate throughout the test, 77% of the initial concentration was maintained. It was deemed more appropriate to prepare individual treatment solutions by adding the test substance to dilution water and removing the WAF of each mixture for testing, rather than preparing dilutions of a stock solution as outlined in the guideline.</p>									
<p>Results:</p> <p>Units/Value:</p> <p>Note: Analytical method, biological observations, control survival.</p>	<p>The maximum actual loading rate causing no mortality after 96 hours was 0.47 mg/L. The minimum actual loading rate causing 100% mortality after 96 hours was 1.8 mg/L. The maximum measured concentration causing no mortality after 96 hours was 0.40 mg/L. The minimum measured concentration causing 100% mortality after 96 hours was 1.7 mg/L.</p> <p>Lethal Loading (LL₅₀) / Lethal Concentration (LC₅₀) Values (mg/L)</p> <table border="1"> <thead> <tr> <th></th> <th>LL₅₀</th> <th>LC₅₀</th> </tr> </thead> <tbody> <tr> <td>3 hours</td> <td>>7.0*</td> <td>>6.3*</td> </tr> <tr> <td>6 hours</td> <td>6.8 (CNC)</td> <td>6.2 (CNC)</td> </tr> </tbody> </table>		LL ₅₀	LC ₅₀	3 hours	>7.0*	>6.3*	6 hours	6.8 (CNC)	6.2 (CNC)
	LL ₅₀	LC ₅₀								
3 hours	>7.0*	>6.3*								
6 hours	6.8 (CNC)	6.2 (CNC)								

	<p>24 hours 2.7 (2.2-3.2) 2.7 (2.2-3.3)</p> <p>48 hours 1.8 (1.5-2.2) 1.7 (1.4-2.1)</p> <p>72 hours 1.2 (1.1-1.3) 1.1 (1.0-1.2)</p> <p>96 hours 1.1 (1.0-1.3) 1.0 (0.9-1.2)</p> <p>* Not a calculated value, 8% mortality was observed in the highest loading rate/concentration tested.</p> <p>Values in parentheses are 95% confidence intervals.</p> <p>CNC = Could Not Calculate</p> <p>The method of analysis was automated static headspace gas chromatography with flame ionization detection (HS GC-FID).</p> <p style="text-align: center;">Summary of In-Life observations - % Mortality</p> <table border="1"> <thead> <tr> <th>Loading Rate (mg/L)</th> <th>Control</th> <th>0.47</th> <th>0.90</th> <th>1.8</th> <th>3.5</th> <th>7.0</th> </tr> <tr> <th>Meas. Conc. (mg/L)</th> <td>0</td> <td>0.40</td> <td>0.79</td> <td>1.7</td> <td>3.7</td> <td>6.3</td> </tr> </thead> <tbody> <tr> <td>3 hours</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>8</td> </tr> <tr> <td>6 hours</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>67</td> </tr> <tr> <td>24 hours</td> <td>0</td> <td>0</td> <td>0</td> <td>8</td> <td>92</td> <td>100</td> </tr> <tr> <td>48 hours</td> <td>0</td> <td>0</td> <td>0</td> <td>50</td> <td>100</td> <td>100</td> </tr> <tr> <td>72 hours</td> <td>0</td> <td>0</td> <td>8</td> <td>100</td> <td>100</td> <td>100</td> </tr> <tr> <td>96 hours</td> <td>0</td> <td>0</td> <td>17</td> <td>100</td> <td>100</td> <td>100</td> </tr> </tbody> </table>	Loading Rate (mg/L)	Control	0.47	0.90	1.8	3.5	7.0	Meas. Conc. (mg/L)	0	0.40	0.79	1.7	3.7	6.3	3 hours	0	0	0	0	0	8	6 hours	0	0	0	0	0	67	24 hours	0	0	0	8	92	100	48 hours	0	0	0	50	100	100	72 hours	0	0	8	100	100	100	96 hours	0	0	17	100	100	100
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72 hours	0	0	8	100	100	100																																																			
96 hours	0	0	17	100	100	100																																																			
Conclusion:	After <i>Oncorhynchus mykiss</i> were exposed to WAFs prepared from Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation) for 96 hours, the LL ₅₀ was 1.1 mg/L and the LC ₅₀ was 1.0 mg/L.																																																								
Reliability:	1-Reliable without restrictions.																																																								
Reference:	ExxonMobil Biomedical Sciences, Inc. 2004. FISH, ACUTE TOXICITY TEST on PYROLYSIS C10+ FUEL OIL (FROM PYROLYSIS GASOLINE DISTILLATION). Study # 176958. Unpublished report.																																																								

Fish Acute Toxicity

Test Substance:	<p>Industry Stream Name: Heavy Pyrolysis Fuel Oil</p> <p><u>CAS Number:</u> <u>CAS Inventory Name:</u> 68513-69-9 Residues, petroleum, steam-cracked light 64741-62-4 Clarified oils, petroleum, catalytic cracked 69013-21-4 Fuel oil, pyrolysis 8002-05-9 Petroleum</p> <p>In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is quenched by injection of recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil.</p>
Method/Guideline:	OECD Guideline 203
Year (guideline):	1992
Type (test type):	Fish Acute Toxicity Test
GLP (Y/N):	Yes
Year (study performed):	2003
Species:	<i>Oncorhynchus mykiss</i>
Analytical Monitoring:	Yes
Exposure Period:	96 hours
Statistical Method:	<p>The 24 - 96 hour LL₅₀ and LC₅₀ values were determined using a Trimmed Spearman-Karber Method (Hamilton <i>et al.</i>, 1977).</p> <p>Hamilton, M., R. Russo, R. Thurston, 1977. Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. <i>Environmental Science and Technology</i>, Vol. 11, No. 7, p.714-719.</p>
Test Conditions: <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, volume, replication, environmental conditions, organisms 	<p>Individual Water Accommodated Fractions (WAF's) were prepared for each treatment. The test substance was added to 18 L of reconstituted water in glass aspirator bottles (capacity 22 L). The solutions were mixed for 24 hours using a 3% vortex (of the static liquid depth). The test solutions were removed through the outlet at the bottom of each mixing vessel into three replicates of approximately 4.5</p>

<p>supplier, loading, deviations from guideline or protocol.</p>	<p>L in 4 L size aspirator bottles (no headspace). Four fish were added to each replicate and the replicates were closed with foil covered neoprene stoppers. Daily renewals were performed by removing ~90% of the test solution through the outlet at the bottom of the aspirator bottle and refilling with fresh solution. The fish were received from Thomas Fish Company, Anderson, CA. The fish were not fed during the study. They were held for 12 days in study dilution water prior to use and were 36 days old at the start of the study. Fish mean weight = 0.194 g, mean total length = 3.1 cm, test loading = 0.172 g of fish/L.</p> <p>Mean test temperature: 13.6°C (S.D. = 0.1), diurnal light: approximately 16 hours light and 8 hours dark with 644 to 653 Lux during full daylight periods. Dissolved oxygen ranged from 6.7 to 8.5 mg/L and pH ranged from 6.5 to 8.0 during the study. Water hardness was 98 mg/L as CaCO₃.</p> <p>Due to the complex nature and limited water solubility of the test substance, the following exceptions to the guideline apply for this study:</p> <p>The concentration of the test substance in solution was not determined prior to use. It was deemed more appropriate to prepare individual treatment solutions by adding the test substance to dilution water and removing the WAF of each mixture for testing than to prepare dilutions of a stock solution.</p> <p>The protocol required that the fish would be held at test temperature (13-15°C) for at least 7 days prior to use in the test. The fish were held at 12.8°C for the 7 days prior to use in the study. This deviation is not believed to have affected the outcome or integrity of the study.</p>															
<p>Results: Units/Value: Note: Analytical method, biological observations, control survival.</p>	<p>The maximum actual loading rate causing no mortality after 96-hours was 2.6 mg/L. The maximum measured concentration causing no mortality after 96-hours was 2.5 mg/L. The minimum actual loading rate causing 100% mortality after 96-hours was 11 mg/L. The minimum measured concentration causing 100% mortality after 96-hours was 9.1 mg/L. The method of analysis was gas chromatography with flame ionization detection (GC-FID).</p> <p>Lethal Loading (LL₅₀) / Lethal Concentration (LC₅₀) Values (mg/L)</p> <table border="1"> <thead> <tr> <th></th> <th>LL₅₀</th> <th>LC₅₀</th> </tr> </thead> <tbody> <tr> <td>3 & 6 hours</td> <td>>11*</td> <td>>9.1*</td> </tr> <tr> <td>24 hours</td> <td>7.5 (6.7-8.4)</td> <td>5.8 (5.2-6.4)</td> </tr> <tr> <td>48 hours</td> <td>5.9 (4.8-7.3)</td> <td>4.7 (3.9-5.6)</td> </tr> <tr> <td>72 & 96 hours</td> <td>5.6 (4.5-6.9)</td> <td>4.4 (3.7-5.3)</td> </tr> </tbody> </table> <p>* Not a calculated value, no mortality was observed in the highest loading rate/concentration at 3 hours, 42% mortality</p>		LL ₅₀	LC ₅₀	3 & 6 hours	>11*	>9.1*	24 hours	7.5 (6.7-8.4)	5.8 (5.2-6.4)	48 hours	5.9 (4.8-7.3)	4.7 (3.9-5.6)	72 & 96 hours	5.6 (4.5-6.9)	4.4 (3.7-5.3)
	LL ₅₀	LC ₅₀														
3 & 6 hours	>11*	>9.1*														
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72 & 96 hours	5.6 (4.5-6.9)	4.4 (3.7-5.3)														

	<p>was observed in the highest loading rate/concentration at 6 hours therefore the EL/EC₅₀ is greater than the highest loading rate/concentration tested. Values in parentheses are 95% confidence intervals.</p> <p style="text-align: center;">Summary of In-Life observations - % Mortality</p> <table border="1"> <thead> <tr> <th>Loading Rate (mg/L)</th> <th>Control</th> <th>0.63</th> <th>1.4</th> <th>2.6</th> <th>5.8</th> <th>11</th> </tr> <tr> <th>Meas. Conc. (mg/L)</th> <th>0</th> <th>0.30</th> <th>1.2</th> <th>2.5</th> <th>4.1</th> <th>9.1</th> </tr> </thead> <tbody> <tr> <td>3 hours</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> </tr> <tr> <td>6 hours</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>42</td> </tr> <tr> <td>24 hours</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>8</td> <td>100</td> </tr> <tr> <td>48 hours</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>42</td> <td>100</td> </tr> <tr> <td>72 & 96 hours</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>58</td> <td>100</td> </tr> </tbody> </table>	Loading Rate (mg/L)	Control	0.63	1.4	2.6	5.8	11	Meas. Conc. (mg/L)	0	0.30	1.2	2.5	4.1	9.1	3 hours	0	0	0	0	0	0	6 hours	0	0	0	0	0	42	24 hours	0	0	0	0	8	100	48 hours	0	0	0	0	42	100	72 & 96 hours	0	0	0	0	58	100
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Conclusion:	After <i>Oncorhynchus mykiss</i> were exposed to WAFs prepared from Heavy Pyrolysis Fuel Oil for 96-hours, the LL ₅₀ was 5.6 mg/L and the LC ₅₀ was 4.4 mg/L.																																																	
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Fish Acute Toxicity

<u>Test Substance</u>	No. 2 fuel oil; CAS # 68476-30-2
<u>Method</u>	
Method/guideline followed	EPA/600/4-90/027 (Weber, C.I., 1991)
Year (guideline)	1991
Type (test type)	Static Renewal Fish Acute Toxicity – WAF
GLP	Yes
Year (study performed)	1998
Species	Sheepshead minnow (<i>Cyprinodon variegates</i>) purchased from Aquatic Biosystems Inc., Ft. Collins, CO
Analytical Monitoring	Yes. Bioavailable Petroleum Hydrocarbons (BPH); Total Petroleum Hydrocarbons (TPH)
Exposure Period	96 hrs. ± 1 hr.
Statistical Methods	48 hr. LL50 calculated by graphical method; 72 hr., 96 hrLL50 calculated by Trimmed Spearman Karber Method. Nominal loading rates were used in statistical analysis
<u>Test Conditions</u>	
Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, supplier of organisms, age, size, weight, loading	Individual test material exposure solutions were prepared as water accommodated fractions (WAF) by adding No. 2 fuel oil volumetrically by syringe to 4 L of dilution water. Syringe weights were recorded before and after addition of test material to determine actual test material loading rates. Dilution water was natural seawater (~ 20ppt). Solutions were mixed at a vortex of ~20% static liquid depth for 24 hrs ± 1hr., and allowed to settle for approx. 2 hrs, after which time, the WAF was removed. Test vessels were 500 ml glass I-Chem jars with Teflon [®] lined caps (no headspace). Approx. 80% of each solution was renewed daily from a freshly prepared WAF. Three replicates, each with 5 fish/jar, were tested at nominal test material loading levels of 0 (control), 10, 30, 60, and 120 mg/L. Fish were 11 days old at study initiation; weight and length were not specified. Test temperature was 24.8 ⁰ ±0.3 ⁰ C. Light intensity was approx. 69 foot-candles during daylight periods (16 hr. light /8 hr. dark periods). Samples were taken of each new WAF on day 0 for TPH analysis and on days 0, 1, 2, and 3, for BPH analysis. For BPH analysis, samples were mixed (minimal headspace) in vessels containing SPME (solid phase microextraction) fibers overnight. The moles of hydrocarbons that partition to the fibers were quantified using GC/FID (Parkerton, T.F. and Stone, M.A. 1996.

<p><u>Results</u> Units/Value: Note: Deviations from protocol or guideline, analytical method, biological observations, control survival</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>Presentation at SETAC, Nov. 17-21, 1996, Washington DC).</p> <p>24 hr. LL50 was not determined; 48 hr. LL50 = 95 mg/L; 72 hr LL50 = 74 mg/L, 96 hr. LL50 = 56 mg/L. Percent mortality at 96 hrs. was 0, 0, 0, 60, 100% for untreated controls (0) and nominal loading rates of 10, 30, 60, and 120 mg/L respectively. Avg. actual loading rates were 0, 9.9, 32, 60, and 126 mg/L. Avg. BPH levels were 20.6, 88.1, 186.2, 192.4, and 257.4 nmoles/0.26 mg carbon while TPH levels were 0.111, 0.022, 0.048, 0.035, and 0.199 mg/L at 0, 10, 30, 60, and 120 mg/L respectively.</p> <p>96 hr. LL50 = 56 mg/L (95% CI 47-67) based on nominal values.</p> <p>1. Reliable without restriction</p> <p>Targia, M.E. and Freeman, J.J. 1998. No. 2 fuel oil: Sheepshead minnow acute toxicity test. Study no. 142961. Exxon Biomedical Sciences Inc. East Millstone, NJ for Exxon Chemicals Intermediates, Houston, TX, USA. Unpublished report.</p>
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Fish Acute Toxicity

<p><u>Test Substance</u></p> <p><u>Method</u> Method/guideline followed Year (guideline) Type (test type) GLP Year (study performed) Species Analytical Monitoring Exposure Period Statistical Methods</p>	<p>No. 2 fuel oil; CAS #68476-30-2. no compositional data</p> <p>EPA/600/4-90/027 (Weber, C.I., 1991) 1991 Static Renewal Fish Acute Toxicity – WAF Yes 1998 <i>Menidia beryllina</i>, purchased from Aquatic Biosystems Inc., Ft. Collins, CO Yes. Bioavailable Petroleum Hydrocarbons (BPH); Total Petroleum Hydrocarbons (TPH) 96 hrs. ± 1 hr.</p> <p>48 hr. LL50 calculated by maximum likelihood analysis, 72 hr., 96 hr LL50 calculated by Trimmed Spearman Karber Method. Nominal loading rates were used in statistical analysis</p>
<p><u>Test Conditions</u> Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, supplier of organisms, age, size, weight, loading</p>	<p>Individual test material exposure solutions were prepared as water accommodated fractions (WAF) by adding No. 2 fuel oil volumetrically by syringe to 4 L of dilution water. Syringe weights were recorded before and after addition of test material to determine actual test material loading rates. Dilution water was natural seawater (~ 20ppt). Solutions were mixed at a vortex of ~20% static liquid depth for 24 hrs ± 1hr., and allowed to settle for approx. 2 hrs, after which time, the WAF was removed. Test vessels were 500 ml glass I-Chem jars with Teflon[®] lined caps (no headspace). Approx. 80% of each solution was renewed daily from a freshly prepared WAF. Three replicates, each with 5 fish/jar, were tested at nominal test material loading levels of 0 (control), 1.25, 2.5, 5.0, and 10.0 mg/L. Fish were 11 days old at study initiation; weight and length were not specified. Test temperature was 24.8⁰±0.3⁰C. Light intensity was approx. 69 foot-candles during daylight periods (16 hr. light /8 hr. dark periods). Samples were taken of each new WAF on day 0 for TPH analysis and on days 0, 1, 2, and 3, for BPH analysis. For BPH analysis, samples were mixed (minimal headspace) in vessels containing SPME (solid phase microextraction) fibers overnight. The moles of hydrocarbons that partition to the fibers were quantified</p>

<p><u>Results</u> Units/Value: Note: Deviations from protocol or guideline, analytical method, biological observations, control survival</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>using GC/FID (Parkerton, T.F. and Stone, M.A. 1996. Presentation at SETAC, Nov. 17-21, 1996, Washington DC).</p> <p>24 hr. LL50 was not determined; 48 hr. LL50 = 4.5 mg/L; 72 hr LL50 = 3.2 mg/L, 96 hr. LL50 = 3.2 mg/L. Percent mortality at 96 hrs. was 0, 0, 13, 100, and 100% for untreated control (0) and nominal loading rates of 0, 1.25, 2.5, 5.0 and 10.0 mg/L respectively. Avg. actual loading rates were 0, 1.5, 2.9, 5.2, and 10 mg/L. Avg. BPH levels were 20.6 (control), 37.2, 50.3, 78.1, and 114.4 nmoles/0.26 mg carbon at 1.25, 2.5, 5.0, and 10.0 mg/L respectively. TPH levels were 0.005, 0.009, and 0.018 mg/L at 1.25, 2.5, and 10.0 mg/L respectively. No TPH level was reported at 5.0 mg/L.</p> <p>96 hr. LL50 = 3.2 mg/L (95% CI 2.9 – 3.6) based on nominal values.</p> <p>1. Reliable without restriction</p> <p>Targia, M.E. and Freeman, J.J. 1998. No. 2 fuel oil: Fish acute toxicity test with <i>Menedia beryllina</i>. Study no. 1422940MB. Exxon Biomedical Sciences Inc. East Millstone, NJ for Exxon Chemicals Intermediates, Houston, TX, USA. Unpublished report.</p>
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	<p>with no aeration.</p> <p>Mean test temperature: 20.1°C (S.D. = 0.1), diurnal light: approximately 16 hours light and 8 hours dark with 91 to 135 lux during full daylight periods. Dissolved oxygen ranged from 7.9 to 8.1 mg/L and pH ranged from 8.1 to 8.3 during the study. Water hardness was 144 mg/L as CaCO₃.</p> <p>The daphnids were cultured in-house. Age was <24 hours old from 15-day old parents.</p> <p>Due to the relatively complex nature and limited water solubility of the test substance, the following exceptions to the guideline apply for this study: The concentration of the test substance in solution was not determined prior to use. It was deemed more appropriate to prepare individual treatment solutions by adding the test substance to dilution water and removing the WAF of each mixture for testing, rather than preparing dilutions of a stock solution as outlined in the guideline.</p>																																							
<p>Results:</p> <p>Units/Value:</p> <p>Note: Analytical method, biological observations, control survival.</p>	<p>Effect Loading (EL₅₀) / Effect Concentration (EC₅₀) Values (mg/L)</p> <table border="1" data-bbox="618 947 1252 1056"> <thead> <tr> <th></th> <th>EL₅₀</th> <th>EC₅₀</th> </tr> </thead> <tbody> <tr> <td>24 hours</td> <td>2.7 (1.8-4.1)</td> <td>2.7 (1.7-4.2)</td> </tr> <tr> <td>48 hours</td> <td>1.2 (0.83-1.8)</td> <td>1.2 (0.82-1.7)</td> </tr> </tbody> </table> <p>Values in parentheses () are 99% confidence intervals.</p> <p>The maximum actual loading rate causing no immobilization after 48 hours was 0.83 mg/L. The minimum actual loading rate causing 100% immobilization after 48 hours was 1.8 mg/L.</p> <p>The maximum measured concentration causing no immobilization after 48 hours was 0.82 mg/L. The minimum measured concentration causing 100% immobilization after 48 hours was 1.7 mg/L.</p> <p>The method of analysis was automated static headspace gas chromatography with flame ionization detection (HS GC-FID).</p> <table border="1" data-bbox="618 1549 1252 1875"> <thead> <tr> <th rowspan="2">Loading Rate (mg/L)</th> <th rowspan="2">Measured Conc. (mg/L)</th> <th colspan="2">% Immobilization</th> </tr> <tr> <th>24-hour</th> <th>48-hour</th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>0</td> <td>0</td> <td>0</td> </tr> <tr> <td>0.17</td> <td>0.07</td> <td>0</td> <td>0</td> </tr> <tr> <td>0.33</td> <td>0.14</td> <td>0</td> <td>0</td> </tr> <tr> <td>0.83</td> <td>0.82</td> <td>0</td> <td>0</td> </tr> <tr> <td>1.8</td> <td>1.7</td> <td>0</td> <td>100</td> </tr> <tr> <td>4.1</td> <td>4.2</td> <td>100</td> <td>100</td> </tr> </tbody> </table>		EL ₅₀	EC ₅₀	24 hours	2.7 (1.8-4.1)	2.7 (1.7-4.2)	48 hours	1.2 (0.83-1.8)	1.2 (0.82-1.7)	Loading Rate (mg/L)	Measured Conc. (mg/L)	% Immobilization		24-hour	48-hour	Control	0	0	0	0.17	0.07	0	0	0.33	0.14	0	0	0.83	0.82	0	0	1.8	1.7	0	100	4.1	4.2	100	100
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Conclusion:	After <i>Daphnia magna</i> were exposed to WAFs prepared from Pyrolysis C10+ Fuel Oil (from Pyrolysis Gasoline Distillation) for 48 hours, the EL ₅₀ and EC ₅₀ was 1.2 mg/L.
Reliability:	1-Reliable without restrictions.
Reference:	ExxonMobil Biomedical Sciences, Inc. 2004. <i>Daphnia sp.</i> , ACUTE IMMOBILIZATION TEST on PYROLYSIS C10+ FUEL OIL (FROM PYROLYSIS GASOLINE DISTILLATION). Study # 176942. Unpublished report.

Daphnid Acute Toxicity

Test Substance:	<p>Industry Stream Name: Heavy Pyrolysis Fuel Oil</p> <p><u>CAS Number:</u> <u>CAS Inventory Name:</u> 68513-69-9 Residues, petroleum, steam-cracked light 64741-62-4 Clarified oils, petroleum, catalytic cracked 69013-21-4 Fuel oil, pyrolysis 8002-05-9 Petroleum</p> <p>In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is quenched by injection of recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil.</p>
Method/Guideline:	OECD Guideline 202
Year (guideline):	1984
Type (test type):	Daphnid Acute Toxicity Test
GLP (Y/N):	Yes
Year (study performed):	2003
Species:	<i>Daphnia magna</i> Straus
Analytical Monitoring:	Yes
Exposure Period:	48 hours
Statistical Method:	<p>The 24-hour EL₅₀ and EC₅₀ values were determined using a Trimmed Spearman-Kärber Method (Hamilton <i>et al.</i>, 1977). A Binomial Method (Stephan, 1977) was used to determine the 48-hour EL₅₀ and EC₅₀ values.</p> <p>Hamilton, M., R. Russo, R. Thurston, 1977. Trimmed Spearman-Kärber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. <i>Environmental Science and Technology</i>, Vol. 11, No. 7, p.714-719.</p> <p>Stephan, C. E., Methods for Calculating an LC₅₀, <i>Aquatic Toxicology and Hazard Evaluation</i>, ASTM STP 634, F. L. Mayer and J. L. Hamelink, Eds., American Society for Testing and Materials, 1977, pp. 65-84.</p>

<p>Test Conditions:</p> <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, volume, replication, environmental conditions, organisms supplier, loading, deviations from guideline or protocol. 	<p>Individual Water Accommodated Fractions (WAF's) were prepared for each treatment. The test substance was added to 4.0 L of reconstituted water in glass aspirator bottles (capacity 4.5 L). The solutions were mixed for 24 hours using a 5% vortex (of the static liquid depth). The test solutions were removed through the outlet at the bottom of each mixing vessel into four replicates of approximately 140 mL in 125 mL Erlenmeyer flasks (no headspace). Five daphnids were added to each replicate and the replicates were closed. The test was performed under static conditions with no aeration.</p> <p>Mean test temperature: 20.1°C (S.D. = 0.1), diurnal light: approximately 16 hours light and 8 hours dark with 100 to 113 lux during full daylight periods. Dissolved oxygen ranged from 8.0 to 8.6 mg/L and pH ranged from 7.8 to 8.1 during the study. Water hardness was 134 mg/L as CaCO₃.</p> <p>The Daphnids were cultured in-house. Age was <24 hours old from 13-day old parents.</p> <p>Due to the relatively complex nature and limited water solubility of the test substance, the following exceptions to the guideline apply for this study: The concentration of the test substance in solution was not determined prior to use. The initial concentration of the test substance was not maintained at 80% in the lowest loading rate throughout the test, 74% of the initial concentration was maintained. It was deemed more appropriate to prepare individual treatment solutions by adding the test substance to dilution water and removing the WAF of each mixture for testing than to prepare dilutions of a stock solution.</p>									
<p>Results:</p> <p>Units/Value:</p> <p>Note: Analytical method, biological observations, control survival.</p>	<p>Effect Loading (EL₅₀) / Effect Concentration (EC₅₀) Values (mg/L)</p> <table border="1"> <thead> <tr> <th></th> <th>EL₅₀</th> <th>EC₅₀</th> </tr> </thead> <tbody> <tr> <td>24 hours</td> <td>3.7 (3.3-4.2*)</td> <td>3.0 (2.7-3.4*)</td> </tr> <tr> <td>48 hours</td> <td>3.3 (2.3-4.8**)</td> <td>2.7 (1.8-4.1**)</td> </tr> </tbody> </table> <p>* 95% Confidence Interval ** 99% Confidence Interval</p> <p>The maximum actual loading rate causing no immobilization after 48-hours was 2.3 mg/L. The minimum actual loading rate causing 100% immobilization after 48 hours was 4.8 mg/L.</p> <p>The maximum measured concentration causing no immobilization after 48-hours was 1.8 mg/L. The minimum measured concentration causing 100% immobilization after 48-hours was 4.1 mg/L.</p> <p>The method of analysis was gas chromatography with flame</p>		EL ₅₀	EC ₅₀	24 hours	3.7 (3.3-4.2*)	3.0 (2.7-3.4*)	48 hours	3.3 (2.3-4.8**)	2.7 (1.8-4.1**)
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Daphnid Acute Toxicity

<u>Test Substance</u>	Biphenyl feedstock, CAS #68989-41-3
<u>Method</u>	
Method/guideline followed	None specified, but comparable to an OECD 202 standard method.
Year (guideline)	NA
Type (test type)	Acute Daphnid Flow-through toxicity study
GLP	Yes
Year (study performed)	1984
Species	Water flea (<i>Daphnia magna</i>) from Sea Plantations, Inc., Salem, MA
Analytical Monitoring	Yes, Total organic carbon (TOC)
Exposure Period	48 hrs
Statistical Methods	Probit procedure of SAS. Chi square on each dose-response curve to verify non-heterogeneity and goodness of fit.
<u>Test Conditions</u>	
Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, supplier of organisms, age, size, loading	First instar (24-hr old) offspring of 20 gravid Daphnia were used as test animals in a flow-through proportional diluter system. One day prior to introduction into the flow-through system, 1000mg/l nominal concentration of Biphenyl feedstock was prepared by mixing 14.4g of test material into 14.4l water. Stock solution was continuously stirred while being dispensed into the diluter. Test concentrations of 0, 10, 18, 32, 56, 100 mg/l (nominal) were produced by dilution with volumes of control water. Flow rate through the system was 14.4l of test preparation dispensed every 24 hrs (25ml dispensed during each 2.5 min/cycle. The flow-through system delivered 100 ml test solution to each dose level every 2.5 min/cycle (50ml into each 3.0 liter capacity duplicate flask). Starting at one end of the bioassay table, 2 Daphnia were placed into a floating polypropylene cylindrical container with a mesh bottom in each test vessel (2 vessels/dose) in consecutive order to the end of the table, procedure was repeated going in reverse direction, then back again until each vessel contained 10 Daphnia. Use of the cylindrical container in the test vessel allowed water to pass through, improved observation of the animals and prevented Daphnia from escaping and being accidentally discharged at the overflow standpipe. All acclimation and test vessels contained charcoal filtered municipal water, pH 7.8-8.5, at a temperature range of 18.4-20.3 ⁰ C and daily photoperiod of a

<p><u>Results</u> Units/Value: Note: Deviations from protocol or guideline, analytical method, biological observations, control survival</p> <p><u>Conclusions</u></p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>Reference</u></p>	<p>continuous 12-hr light/dark cycle. Daily records were kept on number of immobilized and dead animals/vessel at 6, 24, and 48 hrs. Water temperature was recorded daily. Dissolved oxygen conc. (9.3-10.1 mg/l), pH, total alkalinity (31 mg/l as CaCO₃) specific conductivity (297-311 umhas/om), and hardness (80-82 mg/l as CaCO₃) were measured during the first 6 hrs, and at 48 hrs in each vessel. Water samples for chemical analysis of actual concentration by fluorescence spectrometer (410nm wavelength) were collected on day 0 and at 48 hrs from each bioassay vessel. Actual concentration was calculated by subtracting the mean background TOC value of 6.8mg/l from analytical test material concentrations at each dose level. The positive control compound, hexavalent chromium as potassium dichromate was detected in water samples by atomic absorption spectrometry.</p> <p>EL₅₀(24-hr) = >100 mg/l (95% Confidence limits not determined) EL₅₀(48-hr) = 23.6 mg/l (95% C.L. 17.5 - 30.7 mg/l) based on nominal values</p> <p>Percent mortality at 48 hrs was 0, 22, 39, 44, 89, and 100% for untreated control (0), and nominal concentrations of 10, 18, 32, 56, 100 mg/l, respectively. Actual water soluble concentrations of biphenyl feedstock were 0.1, 1.7, 2.7, 3.8, and 7.5 mg/l at nominal concentrations of 10, 18, 32, 56, 100 mg/l, respectively. No treatment related changes were induced in any water characteristic measurement by the test material or positive control. The 24-hr and 48-hr EC₅₀ values for hexavalent chromium were 0.18mg/l and 0.13mg/l, respectively.</p> <p>The 48-hr EL₅₀ for Biphenyl feedstock was 23.6mg/l, based on nominal values. Analytical results revealed that the maximum saturated test concentration achievable was 7.5mg/l.</p> <p>1. Reliable without restriction.</p> <p>Meyers, W.R., Rausina, G.A. 1984. 48-hour Aquatic toxicity study in <i>Daphnia</i> with Biphenyl Feedstock. Proj. #2042. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemical Co., Houston, TX, USA, USA. Unpublished</p>
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	<p>Duncan, D.B. 1975, "t-Tests and Intervals for Comparisons Suggested by the Data", <i>Biometrics</i>, 31, 339-359.</p> <p>Dunnett, C. 1964, "New Tables for Multiple Comparisons With A Control", <i>Biometrics</i>, Vol 20, No. 3, pg 482-491.</p> <p>Shapiro, S.S. and Wilk, M.B. 1965, "n analysis of variance test for normality (complete samples)" <i>Biometrika</i>, 52, pg 591-611.</p>
<p>Test Conditions:</p> <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, volume, replication, environmental conditions, organisms supplier, loading, deviations from guideline or protocol. 	<p>Individual Water Accommodated Fractions (WAF's) were prepared for each treatment. The test substance was added to 4.0 L of algal nutrient medium augmented with sodium bicarbonate in glass aspirator bottles (capacity 4.5 L). The solutions were mixed for 24 hours using a 7% vortex (of the static liquid depth). The test solutions were removed through the outlet at the bottom of each mixing vessel into 12 replicates of approximately 140 mL in 125 mL Erlenmeyer flasks (no headspace) containing two 14 mm glass spheres to facilitate mixing. The test chambers were inoculated with algae (1.0×10^4 cells/mL) and were sealed with ground glass stoppers. Three replicates were sacrificed daily for cell density determination. The test chambers were placed on a shaker table (100 rpm) to keep the algae in suspension. The test was performed under static conditions with no aeration. The algae was cultured in-house from 5 day old stock cultures in log phase growth.</p> <p>Mean test temperature: 24.5°C (sd = 0.3). Continuous light: intensity was 8288 to 8589 Lux. The pH ranged from 7.5 to 7.6 in the test solutions at test initiation and ranged from 7.8 to 9.5 at test termination.</p> <p>Due to the complex nature and limited water solubility of the test substance, the following exceptions to the guideline apply for this study: The concentration of the test substance in solution was not determined prior to use. Test substance analysis was performed on samples of the WAFs at the start of the test (day 0) and at termination (day 4). The initial concentration of the test substance was not maintained at 80% in the three lower loading rates throughout the test (this may be due to biological activity or physical processes in the test chambers). It was appropriate to prepare individual treatment solutions by adding the test substance to dilution water and removing the WAF of each mixture for testing, rather than preparing dilutions of a stock solution as outlined in the guideline. The test duration was 96 hours, instead of 72 hours. However, both 72 and 96-hour endpoints were determined.</p> <p>None of the above exceptions are believed to have affected the outcome, integrity, or quality of the study.</p>

<p>Results:</p> <p>Units/Value:</p> <p>Note: Analytical method, biological observations, control survival.</p>	<p>Effects on growth rate (r) based upon actual loading rates: 72 hr ErL50 = 2.3 mg/L (2.2 - 2.4 mg/L) 96 hr ErL50 = 2.2 mg/L (2.1 - 2.3 mg/L) 72 hr and 96 hour NOELR = 0.18 mg/L</p> <p>Effects on biomass (b) based upon actual loading rates: 72 hr EbL50 = 1.3 mg/L (1.1 - 1.5 mg/L) 96 hr EbL50 = 1.2 mg/L (CNC) 72 hr and 96 hour NOELR = 0.18 mg/L</p> <p>Effects on growth rate (r) based upon measured concentrations: 72 hr ErC50 = 1.7 mg/L (1.6 - 1.8 mg/L) 96 hr ErC50 = 1.6 mg/L (1.5 - 1.7 mg/L) 72 hr and 96 hour NOEC = 0.12 mg/L</p> <p>Effects on biomass (b) based upon measured concentrations: 72 hr EbC50 = 0.95 mg/L (0.80 - 1.1 mg/L) 96 hr EbC50 = 0.91 mg/L (CNC) 72 hr and 96 hour NOEC = 0.12 mg/L</p> <p>Values in parentheses are 95% confidence intervals. CNC = Could Not Calculate</p> <p>The analytical method used was static headspace gas chromatography with flame ionization detection.</p> <table border="1" data-bbox="618 1066 1382 1157"> <thead> <tr> <th colspan="7">Summary of In-Life observations - % Inhibition</th> </tr> <tr> <th>Loading Rate (mg/L)</th> <th>Control</th> <th>0.10</th> <th>0.18</th> <th>0.46</th> <th>1.3</th> <th>3.3</th> </tr> </thead> <tbody> <tr> <td>Meas. Conc. (mg/L)</td> <td>0</td> <td>0.04</td> <td>0.12</td> <td>0.36</td> <td>0.99</td> <td>2.4</td> </tr> </tbody> </table> <p>Based on Growth Rate</p> <table border="1" data-bbox="618 1184 1382 1247"> <tbody> <tr> <td>72 hours</td> <td>n/a</td> <td>0</td> <td>-1.9</td> <td>6.1</td> <td>17</td> <td>84</td> </tr> <tr> <td>96 hours</td> <td>n/a</td> <td>0</td> <td>-2.9</td> <td>1.1</td> <td>18</td> <td>88</td> </tr> </tbody> </table> <p>Based on Biomass</p> <table border="1" data-bbox="618 1274 1382 1337"> <tbody> <tr> <td>72 hours</td> <td>n/a</td> <td>-1.0</td> <td>-1.6</td> <td>26</td> <td>51</td> <td>95</td> </tr> <tr> <td>96 hours</td> <td>n/a</td> <td>-0.3</td> <td>-7.1</td> <td>16</td> <td>60</td> <td>98</td> </tr> </tbody> </table> <p>Negative (-) value indicates a stimulatory effect.</p>	Summary of In-Life observations - % Inhibition							Loading Rate (mg/L)	Control	0.10	0.18	0.46	1.3	3.3	Meas. Conc. (mg/L)	0	0.04	0.12	0.36	0.99	2.4	72 hours	n/a	0	-1.9	6.1	17	84	96 hours	n/a	0	-2.9	1.1	18	88	72 hours	n/a	-1.0	-1.6	26	51	95	96 hours	n/a	-0.3	-7.1	16	60	98
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<p>Conclusions:</p>	<p>Effects on growth rate (r) based upon actual loading rates: 72 hr ErL50 = 2.3 mg/L 96 hr ErL50 = 2.2 mg/L</p> <p>Effects on biomass (b) based upon actual loading rates: 72 hr EbL50 = 1.3 mg/L 96 hr EbL50 = 1.2 mg/L</p> <p>Effects on growth rate (r) based upon measured concentrations: 72 hr ErC50 = 1.7 mg/L 96 hr ErC50 = 1.6 mg/L</p> <p>Effects on biomass (b) based upon measured concentrations: 72 hr EbC50 = 0.95 mg/L</p>																																																	

	96 hr EbC50 = 0.91 mg/L
Reliability:	(1)-Reliable without restriction
Reference:	ExxonMobil Biomedical Sciences, Inc. 2004. ALGA, GROWTH INHIBITION TEST on PYROLYSIS C10+ FUEL OIL (FROM PYROLYSIS GASOLINE DISTILLATION). Study # 176967. Unpublished report.

Alga Toxicity

Test Substance:	<p>Industry Stream Name: Heavy Pyrolysis Fuel Oil</p> <p><u>CAS Number:</u> <u>CAS Inventory Name:</u> 68513-69-9 Residues, petroleum, steam-cracked light 64741-62-4 Clarified oils, petroleum, catalytic cracked 69013-21-4 Fuel oil, pyrolysis 8002-05-9 Petroleum</p> <p>In ethylene plants cracking liquid feedstocks, the cracking furnace effluent (after heat recovery) is quenched by injection of recycled quench oil. This step results in the condensation of higher boiling hydrocarbon compounds that are typically separated from the rest of the furnace effluent as the bottoms of the oil quench tower. Lights are stripped from the excess oils generated from this quench system, resulting in the stream identified here as heavy pyrolysis fuel oil.</p>
Method/Guideline:	OECD Guideline 201
Year (guideline):	1984
Type (test type):	Alga Toxicity Test
GLP (Y/N):	Yes
Year (study performed):	2003
Species:	<i>Pseudokirchneriella subcapitata</i>
Analytical Monitoring:	Yes
Exposure Period:	96 hours
Statistical Method:	<p>The E_bC_{50}, E_rC_{50} and confidence intervals for inhibition of growth/growth rate slope were determined by a probit regression calculation of the probit of the growth inhibition/growth rate slope vs the log of the concentration and associated confidence intervals based on the methods of D. J. Finney (Finney, 1971). Calculations were based on the PROC PROBIT procedure of SAS (SAS, 2002). The NOEC for the E_bC_{50} and E_rC_{50} was based on Multiple Range tests (Duncan, 1975) and (Dunnett, 1964), determined from the GLM procedure of SAS (SAS, 2002). The Shapiro-Wilk (Shapiro-Wilk, 1965) test for normality was used to test if the assumption of normality of the residuals was met; since the residuals were normally distributed the NOEC was based on the estimated values.</p>

	<p>Finney, D.J. 1971. Probit Analysis, 3rd Edition, London: Cambridge University Press.</p> <p>SAS Version 8, SAS Institute, Inc., Cary, NC. 2002.</p> <p>Duncan, D.B. 1975, "t-Tests and Intervals for Comparisons Suggested by the Data", Biometrics, 31, 339-359.</p> <p>Dunnett, C. 1964, "New Tables for Multiple Comparisons With A Control", Biometrics, Vol 20, No. 3, pg 482-491.</p> <p>Shapiro, S.S. and Wilk, M.B. 1965, "n analysis of variance test for normality (complete samples)" Biometrika, 52, pg 591-611.</p>
<p>Test Conditions:</p> <ul style="list-style-type: none"> Note: Concentration preparation, vessel type, volume, replication, environmental conditions, organisms supplier, loading, deviations from guideline or protocol. 	<p>Individual Water Accommodated Fractions (WAF's) were prepared for each treatment. The test substance was added to 2.0 L of algal nutrient medium augmented with sodium bicarbonate in glass aspirator bottles (capacity 2.3 L). The solutions were mixed for 24.5 hours using an 7% vortex (of the static liquid depth). The test solutions were removed through the outlet at the bottom of each mixing vessel into 12 replicates of approximately 140 mL in 125 mL Erlenmeyer flasks (no headspace) containing two 14 mm glass spheres to facilitate mixing. The test chambers were inoculated with algae (1.0×10^4 cells/mL) and were sealed with ground glass stoppers. Three replicates were sacrificed daily for cell density determination. The test chambers were placed on a shaker table (100 rpm) to keep the algae in suspension. The test was performed under static conditions with no aeration. The algae was cultured in-house from 5 day old stock cultures in log phase growth.</p> <p>Mean test temperature: 24.2°C (sd = 0.5). Continuous light: intensity was 8431 to 8595 Lux. The pH ranged from 7.4 to 7.6 in the test solutions at test initiation and ranged from 7.0 to 8.7 at test termination.</p> <p>Due to the complex nature and limited water solubility of the test substance, the following exceptions to the guideline apply for this study: The concentration of the test substance in solution was not determined prior to use. Test substance analysis was performed on samples of the WAFs at the start of the test (day 0) and at termination (day 4). The initial concentration of the test substance was not maintained at 80% in the three lower loading rates throughout the test (this may be due to biological activity or physical processes in the test chambers). It was appropriate to prepare individual treatment solutions by adding the test substance to dilution water and removing the WAF of each mixture for testing than to prepare dilutions of a stock solution. The test duration was 96 hours, instead of 72 hours. However, both 72 and 96-hour endpoints</p>

	<p>were determined. None of the above exceptions are believed to have affected the outcome, integrity, or quality of the study.</p>																																																	
<p>Results: Units/Value: Note: Analytical method, biological observations, control survival.</p>	<p>Effects on growth rate (r) based upon actual loading rates: 72 hr ErL50 = 2.3 mg/L (CNC) 96 hr ErL50 = 2.1 mg/L (CNC) 72 and 96 hr NOELR = 0.39 mg/L</p> <p>Effects on biomass (b) based upon actual loading rates: 72 hr EbL50 = 1.5 mg/L (1.3-1.6 mg/L) 96 hr EbL50 = 1.4 mg/L (1.3-1.6 mg/L) 72 hr NOELR = 0.20 mg/L 96 hr NOELR = 0.39 mg/L</p> <p>Effects on growth rate (r) based upon measured concentrations: 72 hr ErC50 = 2.0 mg/L (CNC) 96 hr ErC50 = 1.8 mg/L (CNC) 72 and 96 hr NOEC = 0.42 mg/L</p> <p>Effects on biomass (b) based upon measured concentrations: 72 and 96 hr EbC50 = 1.3 mg/L (1.2-1.4 mg/L) 72 hr NOEC = 0.07 mg/L 96 hr NOEC = 0.42 mg/L</p> <p>Values in parentheses are 95% confidence intervals. CNC = Could Not Calculate</p> <p>The analytical method used was static headspace gas chromatography with flame ionization detection.</p> <table border="1" data-bbox="618 1220 1382 1310"> <thead> <tr> <th colspan="7">Summary of In-Life observations - % Inhibition</th> </tr> <tr> <th>Loading Rate* (mg/L)</th> <th>Control</th> <th>0.20</th> <th>0.39</th> <th>1.1</th> <th>2.6</th> <th>7.2</th> </tr> </thead> <tbody> <tr> <td>Meas. Conc. † (mg/L)</td> <td>0</td> <td>0.07‡</td> <td>0.42</td> <td>1.1</td> <td>2.1</td> <td>6.4</td> </tr> </tbody> </table> <p>Based on Growth Rate</p> <table border="1" data-bbox="618 1339 1382 1402"> <tbody> <tr> <td>72 hours</td> <td>n/a</td> <td>-2.0</td> <td>0</td> <td>11</td> <td>83</td> <td>97</td> </tr> <tr> <td>96 hours</td> <td>n/a</td> <td>-1.7</td> <td>-2.2</td> <td>7.1</td> <td>86</td> <td>98</td> </tr> </tbody> </table> <p>Based on Biomass</p> <table border="1" data-bbox="618 1432 1382 1495"> <tbody> <tr> <td>72 hours</td> <td>n/a</td> <td>-2.1</td> <td>7.6</td> <td>34</td> <td>92</td> <td>99</td> </tr> <tr> <td>96 hours</td> <td>n/a</td> <td>-1.9</td> <td>1.3</td> <td>31</td> <td>97</td> <td>100</td> </tr> </tbody> </table> <p>* Actual loading rate (weight) of test substance added to the vehicle/dilution water. † Concentration based on mean (Day 0 and Day 4) measured concentrations. ‡ Based on Day 0 only, since the Day 4 sample was below detection limits. Negative(-) value indicates a stimulatory effect.</p>	Summary of In-Life observations - % Inhibition							Loading Rate* (mg/L)	Control	0.20	0.39	1.1	2.6	7.2	Meas. Conc. † (mg/L)	0	0.07‡	0.42	1.1	2.1	6.4	72 hours	n/a	-2.0	0	11	83	97	96 hours	n/a	-1.7	-2.2	7.1	86	98	72 hours	n/a	-2.1	7.6	34	92	99	96 hours	n/a	-1.9	1.3	31	97	100
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Reliability:	(1)-Reliable without restriction
Reference:	ExxonMobil Biomedical Sciences, Inc. 2004. ALGA, GROWTH INHIBITION TEST on HEAVY PYROLYSIS FUEL OIL. Study # 176867. Unpublished report.

Alga Toxicity

<u>Test Substance</u>	Biphenyl feedstock, CAS #68989-41-3
<u>Method</u>	US EPA 600/9-78-018. <i>Selenastrum capricornutum</i> Printz Algal Assay Bottle Test.
Method/guideline followed	1978
Year (guideline)	Algal toxicity
Type (test type)	Yes
GLP	1983
Year (study performed)	Fresh water green algae (<i>Selenastrum capricornutum</i>)
Species	No
Analytical Monitoring	96 hrs
Exposure Period	
Statistical Methods	Probit analysis of SAS. Chi square on dose response curves to verify non-heterogeneity and goodness of fit.
<u>Test Conditions</u>	
Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, age.	Test material at nominal concentrations of 0, 2.8, 27.8, and 277.8mg/l, was added to 60ml algal medium in 250ml Erlenmeyer flasks (3 flasks/dose), followed by addition of alga at initial cell concentration of 1000 cells/ml. All flasks were cultured for 96 hrs under cool white fluorescent light at 400 ft candles, at 25 ⁰ C on a shaker platform set at 110 oscillations/min. At 96 hrs after inoculation, all cultures were counted 4 times with a Coulter counter. Untreated control flasks (3 cultures) were also counted at 48 hrs after inoculation to confirm logarithmic growth. To verify cell viability, concentration of chlorophyll in each culture was determined. Algal cells were filtered from test media; chlorophyll was extracted from the biomass using 90% v/v aqueous acetone solution, and chlorophyll content was analyzed by measuring fluorescence of extract and converting values to cell numbers using a calibration curve. Positive control compound was zinc chloride administered at nominal concentrations of 10, 50, 100, 150µg/l. Samples of algal assay media were taken from each dose group to determine actual soluble test substance concentration/dose, but were stored frozen for 5 months until analysis. At that time, the samples were considered unsuitable for evaluation and analysis was not performed.
<u>Results</u>	
Units/Value:	At 277.8mg/l nominal concentration of biphenyl feedstock,

<p>Measurement (cells/growth) Note: Deviations from protocol or guideline, analytical method, biological observations, control survival</p>	<p>the % variation in average growth rate was less than 50% (36.58% inhibition). 96 hr EC₅₀ = 150.4ug/l for ZnCl₂. Untreated cultures exhibited log phase growth at 48 hrs and 10⁵ cells minimum at 96 hrs. Cell number verification by chlorophyll analysis indicated approximately 2 fold number of algal cells actually counted with the Coulter counter, probably due to use of older cells in establishing the calibration curve; therefore these data are invalid. Actual water-soluble concentrations of Biphenyl feedstock were not determined.</p>
<p><u>Conclusions</u> (study author)</p>	<p>96 hr EC₅₀ > 277.8mg/l based on nominal concentration of Biphenyl Feedstock.</p>
<p><u>Data Quality</u> <i>Reliabilities</i></p>	<p>3. Not reliable. The effect value is excessively higher than expected based on the invertebrate data developed for this product. Analytical determination of water-soluble conc. of biphenyl feedstock solutions not determined. Confirmation of cell count using chlorophyll concentration was not valid, and 96 hr EL₅₀ for biomass not addressed.</p>
<p><u>Reference</u></p>	<p>Bingham, T.S., Rausina, G.A. 1983. 96-hour Inhibition/Stimulation study in algae using Biphenyl Feedstock. Proj. #2043. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemical Co., Houston, TX, USA.</p>