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SYNTHETIC CRUDE OILS CARCINOGENICITY SCREENING TESTS

FINAL REPORT

Covering the Period October 16, 1978 to August 30, 1980

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ABSTRACT

Eight crude oils (Southern Louisiana Petroleum, H. Coal Syncrude, H Coal Fuel Oil, SRC II, Exxon Donor Solvent Liquid, Occidental in situ Shale Oil, Paraho Shale Oil and Geokinetics in situ Shale Oil) were distilled into , or have been received, as four fractions for analysis and screening for biological (mutagenicity and tumor initiating) activity. Results of selected analytical tests have been obtained on the undistilled crude oils and the fractions. Salmonella typhimurium mutation assay and an accelerated tumor initiation-promotion test have been run on the undistilled crude oils and the fractions.

Low boiling (naphtha) fractions of all eight materials showed little or no mutagenicity or skin tumor initiating activity by the two tests used. The higher boiling fractions (gas oils and residues) and the crude oils themselves were mutagenic and exhibited tumor initiation activity. The coal derived fractions were more active by both tests than the shale oil samples, the latter were similar to the petroleum controls. Few differences were apparent in biological activity between coal derived samples of equivalent boiling range among the various coal liquefaction processes, except that the SRC II naphtha sample showed a degree of acute toxicity through skin absorption not exhibited by the other samples. Generally the results agreed closely for the various samples between the salmonella mutation assay with activation and the skin tumor initiation test.

TABLE OF CONTENTS

	<u>Page Number</u>
Abstract	i
Table of Contents	ii
List of Illustrations	iii
List of Tables	iv
Introduction	1
Experimental Procedures	1
Experimental Results	2
Summary and Conclusions	3
Recommendations	4
References	5
Appendices:	
I. Identification of Samples Tested	38
II. Distillation Procedures	40
III. Analytical Testing of Crude Samples and Distillation Cuts	44
IV. Biological Tests	47
A. Ames Test	47
B. Initiation-Promotion Test	49

LIST OF ILLUSTRATIONS

	<u>Page Number</u>
1. 20-Plate Oldershaw Distillation	42
2. Distillation Column Controls	43

LIST OF TABLES

Page Number

I.	Distillation Fractions	6
II.	Distillation Fractions	7
III.	Observed Cut Points	8
IV.	Observed Cut Points	9
V.	Analytical Results - Southern Louisiana Petroleum	10
VI.	Analytical Results - Paraho Shale Oil	11
VII.	Analytical Results - Geokinetics Shale Oil	12
VIII.	Analytical Results - Occidental Shale Oil	13
IX.	Analytical Results - H Coal Syncrude Mode	14
X.	Analytical Results - H Coal Fuel Oil Mode	15
XI.	Analytical Results - SRC II	16
XII.	Analytical Results - Exxon Donor Solvent	17
XIII.	Non-mutagenic Samples	18
XIV.	Mutagenic Samples	19
XV.	Mouse Skin Painting Initiation-Promotion Study	28
XVI.	Comparison of the Salmonella and Initiation-Promotion Test Results - South Louisiana Petroleum	30
XVII.	Comparison of the Salmonella and Initiation-Promotion Test Results - Paraho Shale Oil	31
XVIII.	Comparison of the Salmonella and Initiation-Promotion Test Results - Geokinetics Shale Oil (In Situ)	32
XIX.	Comparison of the Salmonella and Initiation-Promotion Test Results - Occidental Shale Oil (In Situ)	33
XX.	Comparison of the Salmonella and Initiation-Promotion Test Results - H Coal Syncrude Mode	34
XXI.	Comparison of the Salmonella and Initiation-Promotion Test Results - H Coal Fuel Oil Mode	35
XXII.	Comparison of the Salmonella and Initiation-Promotion Test Results - SRC II	36
XXIII.	Comparison of the Salmonella and Initiation-Promotion Test Results - Exxon Donor Solvent Process (Coal)	37

Syncrude Carcinogenicity Screening Tests

INTRODUCTION

This Report is the Final Report for the U. S. Department of Energy Agreement DE-AC02-78EV (formerly EP-78-C-02-4758).

The objective of this project was to define in a preliminary fashion, the carcinogenic potential of materials expected to be encountered in the production, handling and utilization of synthetic crude oils and common distillation fractions derived from them. A crude petroleum and seven synthetic crude oils derived from coal and oil shale by several processes under development were studied. Selected analytical tests were run on the crude oils and distillation fractions to provide some characterization of them. Salmonella mutation tests and mouse skin tumor initiation-promotion tests were conducted on these same materials.

EXPERIMENTAL PROCEDURES

The project was run in two parts. In each part, four crude oils were processed. South Louisiana crude petroleum, H Coal Syncrude, Paraho Shale Oil and Geokinetics in situ Shale Oil were included in the first part. The Southern Louisiana crude petroleum, Paraho Shale Oil and Geokinetics shale oil were received as whole crude and were distilled according to the procedure in ASTM D2892. Fractions were taken in boiling ranges shown in Appendix II. The H Coal syncrude samples arrived as fractions. Since boiling ranges of most of the fractions corresponded roughly with the boiling ranges desired, only the atmosphere still overhead sample was redistilled. Boiling ranges were checked by Simulated Distillation analysis.

The second four syncrudes studied were H Coal fuel oil mode; SRC II; Exxon Donor Solvent (EDS) liquid; and Occidental Petroleum Co. In Situ Shale Oil. The first three of these syncrudes came as fractions. These fractions were tested "as received"; however, in addition, the vacuum gas oil boiling range materials were distilled into two additional fractions.

Details of the distillation equipment and distillation procedure(s) are described in Appendix II. Samples were nitrogen blanketed and, in some cases, sealed in glass ampules. Analytical tests are described in Appendix III.

Details of the biological test protocols are given in Appendix IV.

EXPERIMENTAL RESULTS

Distillation yields on all samples distilled are shown in Tables I and II. Conditions of these distillations and observed cut points are shown in Tables III and IV.

Analytical results on all syncrudes and their fractions are shown in Tables V through XII.

The Salmonella Typhimurium (Ames) mutation test results are shown in Tables XIII and XIV. The average reversion frequencies are presented in Appendix IV.

Mouse skin painting initiation-promotion results are shown in Table XV.

The SRC II naphtha sample, unlike the other naphtha samples tested exhibited acute toxicity through skin absorption at a fairly high dose level (1.6 g/kilogram of body weight). Of the 30 mice painted with this material, 23 died within 48 hours. This test was repeated with 10 additional mice under conditions in which the inhalation of the fumes was minimized. Seven of the 10 mice died. To select a non-toxic concentration, a dose-response study was conducted with samples diluted with toluene. As a further precaution, the mice were treated in a frame hood to minimize inhalation exposure.

DISCUSSION OF RESULTS

The Salmonella mutation and initiation/promotion test data are shown in Tables XVI - XXIII. The Salmonella test data shown on the tables were obtained with an activation system; activities observed without activation are indicated by footnotes. Most of the mutagenic activities were observed when the treatment was conducted in the presence of the activation system. Several samples were mutagenic without activation; however, this activity was generally less than that observed with activation. If a sample exhibited mutagenic activity in a specific strain without activation, it also was mutagenic in the same strains with activation. The greatest activities were in strains TA 1537, TA 1538, and TA 98, which exhibit special sensitivity to frame-shift mutagens. The data obtained with benzo(a)pyrene are included in Table XVI.

In general, the Salmonella mutation test data correlated well with the results from the initiation/promotion test. The crude and high boiling fractions of petroleum, shale oil, and coal liquids exhibited mutagenic and tumor initiation activities as measured in the Salmonella and initiation-promotion tests. The high boiling fractions exhibited the greatest activities in both screening tests. None of the naphtha fractions were considered to be active. The crude shale oils and fractions exhibited biological activities quite similar to those observed with the South Louisiana crude petroleum sample and fractions selected as controls.

The coal derived crude oils and their higher boiling fractions all show a significantly higher level of biological activity than do the shale or petroleum materials. In fact, some fractions appear to be as active as the benzo(a)pyrene controls. The various coal derived samples of similar boiling ranges exhibit similar potency regardless of liquefaction process.

SUMMARY AND CONCLUSIONS

The crude and/or high boiling fractions of petroleum, shale oil, and coal liquids exhibited mutagenic and tumor initiating activities, as measured by the Salmonella and initiation-promotion assays. The biological activities of the shale oil and petroleum fractions were consistently lower than those of the coal derived liquids. The biological activities observed among the various coal liquids and among the various shale oils were similar.

The original coals used in producing the coal liquids we tested were similar (all bituminous and in three out of four cases, Illinois #6): as were the biological activities of the coal liquids derived from them. The effect of the liquefaction process, while not clear from this study, should be investigated as commercial, and replicate PDU* samples are produced and tested.

Since all syncrude samples tested were prepared in non-commercial facilities [(PDU) or pilot plants], there is always a question of whether the materials tested in this study are representative of or similar to the commercial products that will eventually be produced. These data together with data from other studies underway will have to serve as an indication of the biological activity of the commercial samples until those materials become available.

*PDU - Process Development Unit

RECOMMENDATIONS:

1. In this study the Salmonella mutation and mouse initiation-promotion tests were used to identify materials that may initiate skin cancer. However, only long-term skin painting studies can establish the value of these predictive tests for testing synfuel fractions. The qualitative and quantitative reliabilities of these tests can be assessed by long-term studies on representative samples that are strongly active, weakly active, and inactive in the predictive tests.
2. When appropriate samples become available, these rapid predictive tests could be used to investigate:
 1. Liquefaction products made from a single coal by various liquefaction processes.
 2. Liquefaction products from a single process with different coals.
 3. Liquefaction products from a single process and a specific coal produced by varying liquefaction conditions, such as hydrogen consumption, pressure and temperature.

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4. F. C. Collins and V. Lantz, "Evaluation of Laboratory Distillation Apparatus", Industrial and Engineering Chemistry, 18:1, 673 (1946).
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TABLE I

DISTILLATION FRACTIONS

Order of Distillation:	1	2	3	4
Sample of:	South LA Petroleum	H-Coal Syncrude	Paraho Shale Oil	Geokinetics Shale Oil
Obtained from:	Texas A&M	HRI	Development Engg. Co.	Geokinetics
Feed to Still	Whole crude	ASOH	Whole crude	Whole crude
<u>Cuts obtained, weight percent of feed</u>				
Light Ends	0.78	0.56	0.00	0.00
Naphtha	14.87	45.18	4.32	5.63
LGO	30.01	50.13	30.96	52.59
MGO	8.48	0	12.48	17.76
Resid	44.54	3.32	51.65	22.09
Loss	1.32	0.82	0.59	1.94
<u>Feed received in:</u>	New 1 gal. paint can	New 1 & 2 gal. lacquer cans	55 gal. Drum Bent Bung	Used 5 gal. Motor oil can
Samples Forwarded for Analysis and Testing	Crude Naphtha LGO MGO Resid*	ASOH ASB VSOH* Naphtha LGO	Crude* Naphtha LGO MGO Resid*	Crude* Naphtha LGO MGO* Resid*

*Samples in screw-top vials. All others in sealed, evacuated glass ampoules

TABLE II

DISTILLATION FRACTIONS

Order of Distillation	5	6	7	8 Not Distilled
Sample of	In Situ Shale Oil	Exxon Donor Solvent	SRC-II	H-Coal Fuel Oil
Obtained from	Occidental	Exxon, Inc.	Pittsburgh & Midway Coal Co.	Hydrocarbon Research, Inc.
Still to Feed	Whole Crude	Vacuum Gas Oil	Stripper tower Bottoms	Not Distilled
<u>Cuts Obtained, Wt.% of Feed</u>				
Light Ends	0	0	0	-
Naphtha	1.11	0	0	-
LGO	46.94	0	0	-
MGO	13.10	0	0	-
HGO	0	22.94	23.58	-
Resid	37.38	76.32	76.14	-
Loss	1.04	0.74	0.28	-
Water	0.35	0	0	-
<u>Feed Received in:</u>	5 Gal. Lacquer Can	1 Gal. Glass Jugs	5 Gal. Oil Cans	Lacquer Cans
Samples Forwarded for Testing	Crude* Naphtha* LGO MGO* Resid*	Naphtha Solvent Vacuum Gas Oil* HGO* Resid*	Naphtha Fuel Oil Vacuum Tar Bt* Stripper Tower Bottom* HGO* Resid*	ASOH** ASB** VSOH** VSB** VSB from prior set of samples*

*Sample in screw top vials, all others in glass ampoules

**AS OH Atmosphere Still Overhead VSOH Vacuum Still Overhead
 ASB Atmosphere Still Bottoms VSB Vacuum Still Bottoms

TABLE III
OBSERVED CUT POINTS

	(1) <u>South LA Petroleum</u>	(2) <u>H-Coal</u>	(3) <u>Paraho</u>	(4) <u>Geokinetics</u>
<u>Light Ends</u> - Collected in dry ice/acetone bath while naphtha distilled				
<u>Naphtha</u> (IBP to 350°F equivalence)				
Pressure, mm Hg	700.	700.	100.	700.
Temp., °C	172.	173.	116.	172.
<u>LGO</u> (350 to 650°F equivalence)				
Pressure, mm Hg	5.0	5.0	5.0	4.9
Temp., °C	180.	127.	179.	179.
<u>MGO</u> (650 to 850°F equivalence)				
Pressure, mm Hg	1.8	-	3.2	3.6
Temp., °C	194.	-	201.	220.
<u>Resid</u> (850°F plus equivalence)				
Reason to stop Run	Pot reached 330°C	Pot reached 330°C	Pot reached 326°C	Pot reached 320°C

TABLE IV
OBSERVED CUT POINTS

	(5) <u>Occidental Shale Oil</u>	(6) <u>EDS</u>	(7) <u>SRC-II</u>
<u>Light Ends</u> - Collected in dry ice/acetone trap while naphtha distilled			
<u>Naphtha</u> (IBP to 350°F equivalence)			
Pressure, mm Hg	100	-	-
Temp. °C	108	-	-
<u>LGO</u> (350 to 650°F equivalence)			
Pressure, mm Hg	5.8	-	-
Temp. °C	180	-	-
<u>MGO</u> (650 to 850°F equivalence)			
Pressure, mm Hg	1.8	-	-
Temp. °C	193	-	-
<u>HGO</u> (IBP to 950°C equivalence)			
Pressure, mm Hg	-	2.2	1.7
Temp. °C	-	287	218
<u>Resid</u>			
Reason to stop run	Pot reached 310°C Cracking started	Pot reached 314°C Cracking started	Pot reached 300°C Cracking started
(8) *H Coal Fuel Oil Mode was not Distilled			

TABLE V

ANALYTICAL RESULTS

SOUTHERN LOUISIANA PETROLEUM

Sample Description	Crude Oil	Naptha Cut	Light Gas Oil Cut	Medium Gas Oil Cut	Resid
<u>Elemental Analysis</u>					
C	86.39	86.33	85.93	86.80	86.51
H	12.25	14.13	12.86	12.46	11.09
N	0.54	<.01	<.10	0.42	0.67
O	0.26	0.07	0.08	0.10	0.35
S	0.90	0.02	0.42	0.83	0.94
TOTAL	100.34	100.55		100.61	99.56
<u>Simulated Distillation Analysis</u>					
°C					
% Light Ends (0-65)	4	10	0	0	0
% Naptha (65-175)	20	72	0	0	0
% Lt. Gas Oil (175-340)	40	17	84	2	0
% Med. Gas Oil (340-430)	21	1	17	95	11
% Heavy Gas Oil (430-500)	12	0	0	3	60
% Unvolatilized Sample*	22.3	<2.	<2.	<2.	10.8
<u>Density (25°C)</u>	.8953	.7564	.8563	.8995	.9513**

*Quantitative measurement of wt. % of SDA sample remaining in GC vaporizer (unvolatilized) after the analysis.

**By water displacement in Hubbard-Carmick pycnometer.

TABLE VI

ANALYTICAL RESULTS
PARAHO SHALE OIL

Sample Description	Crude Oil	Naphtha Cut	Light Gas Oil Cut	Medium Gas Oil Cut	Resid
<u>Elemental Analysis</u>					
C	84.56	84.82	84.53	84.48	85.93
H	11.37	12.72	11.96	11.31	10.97
N	2.15	1.21	1.27	2.34	2.46
O	1.43	1.10	1.24	0.91	0.76
S	0.48	0.82	0.67	0.61	0.38
TOTAL	99.99	100.67	99.67	99.65	100.50
<u>Simulated Distillation Analysis</u>					
°C					
% Light Ends (0-65)	0	0	0	0	0
% Naphtha (65-175)	0	53	0	0	0
% Lt. Gas Oil (175-340)	32	46	81	5	2
% Med. Gas Oil (340-430)	33	2	19	91	7
% Heavy Gas Oil (430-500)	27	0	0	5	61
% Unvolatilized Sample*	<2.	<2.	<2.	<2.	13.7
<u>Density (25°C)</u>	.9246**	.8024	.8796	.9872**	1.0691**

*Quantitative measurement of wt. % of SDA sample remaining in GC vaporizer (unvolatilized) after the analysis.

**By water displacement in Hubbard-Carmick pycnometer.

TABLE VII
ANALYTICAL RESULTS
GEOKINETICS SHALE OIL

Sample Description	Crude Oil	Naphtha Cut	Light Gas Oil Cut	Medium Gas Oil Cut	Resid
<u>Elemental Analysis</u>					
C	84.71	84.05	85.40	85.33	85.60
H	11.93	13.03	12.15	11.71	11.28
N	1.69	1.40	2.18	1.97	2.07
O	0.81	0.83	0.61	0.39	0.61
S	0.54	0.47	0.42	0.24	0.42
TOTAL	99.68	99.78	100.76	99.64	99.98
<u>Simulated Distillation Analysis °C</u>					
% Light Ends (0-65)	0	0	0	0	0
% Naphtha (65-175)	2	49	0	0	0
% Lt. Gas Oil (175-340)	55	50	87	0	1
% Med. Gas Oil (340-430)	32	1	13	94	7
% Heavy Gas Oil (430-500)	12	1	1	6	76
% Unvolatilized Sample*	<2.	<2.	<2.	<2.	<2.
<u>Density (25°C)</u>	.8898	.7926	.8647	.9159	.9636

*Quantitative measurement of wt. % of SDA sample remaining in GC vaporizer (unvolatilized) after the analysis.

TABLE VIII

ANALYTICAL RESULTS
OCCIDENTAL SHALE OIL

<u>Sample Description</u>	<u>Crude</u>	<u>Naphtha</u>	<u>LGO</u>	<u>MGO</u>	<u>Resid</u>
<u>Elemental Analysis</u>					
C	85.40	84.11	84.69	85.47	85.45
H	12.11	13.01	12.18	12.00	11.94
N	1.66	2.26	1.46	1.58	1.50
O	.94	1.05	.83	.68	.94
S	.74	.87	.55	.52	.61
TOTAL	100.85	101.30	99.71	100.25	100.44
<u>Simulated Distillation Analysis (°C)</u>					
% Light Ends (0-65)	0	0	0	0	0
% Naphtha (65-175)	1	44	0	0	0
% Lt. Gas Oil (175-340)	41	57	84	1	5
% Med. Gas Oil (340-430)	31	0	17	40	0
% Heavy Gas Oil (430-500)	20	0	0	60	78
% Unvolatilized Sample	<2.	<2.	<2.	<2.	~14.8
<u>Density (25°C) g/cc</u>	0.9073	0.8088	0.8760	0.9244**	0.9677

**By water displacement in Hubbard-Carmick Pycnometer

TABLE IX
ANALYTICAL RESULTS
H. COAL SYNCRUDE MODE

Sample Description	Atm. Still Overheads	Naphtha Cut	Light Gas Oil Cut	Atm. Still Bottoms	Vac. Still Overheads	Vac. Still Bottoms
<u>Elemental Analysis</u>						
C	86.82	85.83	88.54	89.58	90.27	66.24
H	11.07	12.66	10.04	8.79	7.89	4.16
N	0.17	0.16	0.17	.17	1.10	1.25
O	1.65	1.40	1.91	1.12	0.99	12.91
S	0.23	0.21	.09	.09	0.12	2.23
<u>TOTAL</u>	<u>99.94</u>	<u>100.26</u>	<u>100.75</u>	<u>99.75</u>	<u>100.37</u>	
<u>Simulated Distillation Analysis</u> °C						
% Light Ends (0-65)	3	5	0	0	0	0
% Naphtha (65-175)	35	72	0	1	0	0
% Lt. Gas Oil (175-340)	61	24	100	70	41	33
% Med. Gas Oil (340-430)	2	0	0	27	49	34
% Heavy Gas Oil (430-500)	0	0	0	3	11	18
% Unvolatilized Sample*	<2.	<2.	<2.	<2.	2.65	89.4
<u>Density (25°C)</u>	.8797	.8107	.9362	1.0089	1.0640	1.41**

*Quantitative measurement of wt. % of SDA sample remaining in GC vaporizer (unvolatilized) after the analysis.

**Helium Pycnometer at 22°C.

TABLE X

ANALYTICAL RESULTS
H-COAL-FUEL OIL MODE

<u>Sample Description</u>	<u>ASOH</u>	<u>ASB</u>	<u>VSOH</u>	<u>VSB</u>
<u>Elemental Analysis</u>				
C	85.58	88.58	88.88	70.89
H	12.02	9.01	8.47	4.56
N	.86	.78	.69	1.28
O	1.45	1.54	1.40	10.84
S	.41	.12	.15	1.81
TOTAL	100.32	100.03	99.59	89.38
<u>% Ash @ 2500°F</u>	-	-	-	17.57
<u>Simulated Distillation Analysis (°C)</u>				
% Light Ends (0-65)	2	0	0	0
% Naphtha (65-175)	50	1	1	0
% Lt. Gas Oil (175-340)	49	74	51	33
% Med. Gas Oil (340-430)	0	24	38	35
% Heavy Gas Oil (430-500)	0	2	10	20
% Unvolatilized Sample, 300°C	2.17	<2,	4.24	~58,2
<u>Density (25°C) g/cc</u>	.8442	.9926	1.031	1.49*

*Helium Pycnometer at 22°C

TABLE XI

ANALYTICAL RESULTS
SRC-II

<u>Sample Description</u>	<u>Naphtha</u>	<u>Oil</u>	<u>HGO</u>	<u>STB**</u>	<u>VTB***</u>	<u>Resid</u>
<u>Elemental Analysis</u>						
C	83.46	86.48	89.38	72.86	67.31	67.70
H	11.12	8.64	7.24	4.68	4.08	3.85
N	.93	1.02	1.09	1.30	1.20	1.16
O	3.44	3.94	1.58	8.94	11.42	11.55
S	.28	.23	.47	2.47	2.69	3.53
TOTAL	99.23	100.31	99.76	90.25	86.70	87.79
% Ash @ 2500°F				17.50	23.88	24.85
<u>Simulated Distillation Analysis (°C)</u>						
% Light Ends (0-65)	6	0	0	0	0	0
% Naphtha (65-175)	64	5	0	0	0	0
% Lt. Gas Oil (175-340)	31	87	31	32	26	30
% Med. Gas Oil (340-430)	0	8	65	47	42	44
% Heavy Gas Oil (430-500)	0	1	5	22	33	27
% Unvolatilized Sample	<2.	<2.	<2.	~27.4	~59.8	~65.8
Density (25°C) g/cc	.8529	.9921	1.099	1.31*	1.46*	1.46*

*Helium Pycnometer at 22°C

**Stripping Tower Bottoms STB

***Vacuum Tower Bottoms VTB

TABLE XII

ANALYTICAL RESULTS
EXXON DONOR SOLVENT

<u>Sample Description</u>	<u>Naphtha</u>	<u>Solvent</u>	<u>VGO</u>	<u>HGO</u>	<u>Resid</u>
<u>Elemental Analysis</u>					
C	84.20	88.58	85.90	87.49	85.84
H	12.04	9.17	6.52	7.21	6.48
N	.46	.45	1.17	1.06	1.31
O	2.37	1.04	3.46	3.18	3.59
S	.60	.23	1.26	.64	1.22
TOTAL	99.67	99.47	98.31	99.58	98.44
% Ash @ 2500°F			.079		.550
<u>Simulated Distillation Analysis (°C)</u>					
% Light Ends (-65)	8	0	0	0	0
% Naphtha (65-175)	59	0	0	0	0
% Lt. Gas Oil (175-340)	34	82	33	17	28
% Med. Gas Oil (340-430)	0	17	38	67	39
% Heavy Gas Oil (430-500)	0	2	30	17	34
% Unvolatilized Sample	<2.	<2.	~15.3	~4.25	~24.7
<u>Density (25°C) g/cc</u>	.8242	.9787	1.17	1.111**	1.15*

*Helium Pycnometer at 22°C

**By water displacement in Hubbard-Carmick Pycnometer

TABLE XIII

Judged on the basis of the statistical criteria outlined in Appendix IV, the following test samples are not mutagenic in the Ames Assay:

South Louisiana Petroleum Naphtha

South Louisiana Petroleum Light Gas Oil

Paraho Shale Oil Naphtha

Paraho Shale Oil Light Gas Oil

Geokinetics in situ Shale Oil Naphtha

Geokinetics in situ Shale Oil Light Gas Oil

Occidental in situ Shale Oil Naphtha

H Coal Syncrude Naphtha

H Coal Syncrude Atmosphere Still Overhead

H Coal Syncrude Light Gas Oil

H Coal Fuel Oil Mode Atmosphere Still Overhead

SRC II Naphtha

Exxon Donor Solvent (EDS) Naphtha

TABLE XIV

Based on the statistical criteria outlined in Appendix IV, the following test samples are mutagenic in the assay:

Name	Strain	Activation System	Activity
Petroleum Fraction, South Louisiana-Mid Gas Oil	TA 1537	+	2.4-2.6 times the spontaneous reversion frequency (0.01 rev/ μ g)
	TA 1538	+	1.8-2.0 times the spontaneous reversion frequency (0.02 rev/ μ g)
	TA 98	+	1.8-2.1 times the spontaneous reversion frequency (0.03 rev/ μ g)
	TA 100	+	1.5-1.7 times the spontaneous reversion frequency (0.07-0.10 rev/ μ g)
Petroleum Fraction, South Louisiana Residue	TA 98	+	1.7-3.0 times the spontaneous reversion frequency (0.01-0.02 rev/ μ g)
	TA 100	+	1.7-2.3 times the spontaneous reversion frequency (0.03-0.04 rev/ μ g)
South Louisiana - Crude	TA 100	+	1.6-2.0 times the spontaneous reversion frequency (0.02 rev/ μ g)
Coal Liquids, H-Coal - Atmospheric Still Bottoms	TA 1537	+	11.0-11.9 times the spontaneous reversion frequency (0.1-0.3 rev/ μ g)
	TA 1538	+	48.5-66.2 times the spontaneous reversion frequency (1.6-2.2 rev/ μ g)

Name	Strain	Activation System	Activity
Shale Oil, Paraho - Residue	TA 100	-	1.3-1.5 times the spontaneous reversion frequency (0.02-0.03 rev/ μ g)
	TA 1537	+	14.7-17.4 times the spontaneous reversion frequency (0.04 rev/ μ g)
	TA 1538	+	24.4-38.1 times the spontaneous reversion frequency (0.6-1.0 rev/ μ g)
	TA 98	+	39.7-41.5 times the spontaneous reversion frequency (0.3-0.5 rev/ μ g)
	TA 100	+	3.6-5.1 times the spontaneous reversion frequency (0.03-0.05 rev/ μ g)
Shale Oil, Paraho - Crude	TA 1537	+	9.0-15.0 times the spontaneous reversion frequency (0.05-0.08 rev/ μ g)
	TA 1538	+	38.2-45.6 times the spontaneous reversion frequency (0.5-0.6 rev/ μ g)
	TA 98	+	29.2-30.2 times the spontaneous reversion frequency (0.6-0.7 rev/ μ g)
	TA 100	+	2.7-2.9 times the spontaneous reversion frequency (0.5 rev/ μ g)
Shale Oil, Geokinetics - Crude	TA 1537	+	3.0-5.6 times the spontaneous reversion frequency (0.02-0.03 rev/ μ g)
	TA 1538	+	4.0-8.0 times the spontaneous reversion frequency (0.1 rev/ μ g)
	TA 98	+	4.4-6.9 times the spontaneous reversion frequency (0.1 rev/ μ g)
	TA 100	+	1.6-1.7 times the spontaneous reversion frequency (0.08-0.09 rev/ μ g)

Name	Strain	Activation System	Activity
Shale Oil, Geokinetics - Mid Gas Oil	TA 1537	-	2.1-3.3 times the spontaneous reversion frequency (0.002-0.003 rey/ μ g)
	TA 98	-	1.9-3.0 times the spontaneous reversion frequency (0.003-0.004 rey/ μ g)
	TA 1537	+	5.5-12.5 times the spontaneous reversion frequency (0.01 rey/ μ g)
	TA 1538	+	9.3-12.8 times the spontaneous reversion frequency (0.07-0.09 rey/ μ g)
	TA 98	+	11.8-25.5 times the spontaneous reversion frequency (0.2 rey/ μ g)
	TA 100	+	2.9-3.0 times the spontaneous reversion frequency (0.2 rey/ μ g)
Shale Oil, Geokinetics, Residue	TA 1537	+	7.2-7.3 times the spontaneous reversion frequency (0.05-0.06 rey/ μ g)
	TA 1538	+	24.9-34.6 times the spontaneous reversion frequency (0.4 rey/ μ g)
	TA 98	+	30.8-34.3 times the spontaneous reversion frequency (0.3 rey/ μ g)
	TA 100	+	3.8-4.4 times the spontaneous reversion frequency (0.3 rey/ μ g)

<u>Note</u>	<u>Strain</u>	<u>Activation System</u>	<u>Activity</u>
H-Coal Fuel Oil Mode; Vacuum Still Bottoms	TA 1538	+	89.2-114.4 times the spontaneous reversion frequency (1.0-3.7 rev/ μ g)
	TA 98	+	60.6-97.8 times the spontaneous reversion frequency (0.57-4.0 rev/ μ g)
	TA 100	+	7.15-27.7 times the spontaneous reversion frequency (0.70-1.7 rev/ μ g)
H-Coal Syncrude Mode; Vacuum Still Bottoms	TA 1537	-	1.8 times the spontaneous reversion frequency (0.014 rev/ μ g)
	TA 1538	-	2.9-9.0 times the spontaneous reversion frequency (0.01-0.07 rev/ μ g)
	TA 98	-	3.0-5.5 times the spontaneous reversion frequency (0.05-0.09 rev/ μ g)
	TA 100	-	1.9-2.4 times the spontaneous reversion frequency (0.14-0.30 rev/ μ g)
	TA 1535	+	2.0-5.5 times the spontaneous reversion frequency (.05-.08 rev/ μ g)
	TA 1537	+	78.9-679.8 times the spontaneous reversion frequency (1.3-1.5 rev/ μ g)
	TA 1538	+	83.0-142.0 times the spontaneous reversion frequency (3.8-5.7 rev/ μ g)
	TA 98	+	111.5-168.1 times the spontaneous reversion frequency (1.3-8.2 rev/ μ g)
	TA 100	+	14.2-24.4 times the spontaneous reversion frequency (1.3-4.1 rev/ μ g)
Occidental Shale Oil; Crude Oil	TA 1537	+	3.4-4.4 times the spontaneous reversion frequency (0.003-0.011 rev/ μ g)
	TA 1538	+	6.8 times the spontaneous reversion frequency (0.036 rev/ μ g)
	TA 98	+	8.2 times the spontaneous reversion frequency (0.03-0.06 rev/ μ g)
	TA 100	+	1.9-3.0 times the spontaneous reversion frequency (0.05-0.13 rev/ μ g)

Name	Strain	Activation System	Activity
Occidental Shale Oil; Light Gas Oil	TA 98	+	2.4-2.5 times the spontaneous reversion frequency (0.04 rev/ μ g)
	TA 100	+	1.5 times the spontaneous reversion frequency (0.07 rev/ μ g)
Occidental Shale Oil, Med. Gas Oil	TA 98	-	2.8 times the spontaneous reversion frequency (0.02 rev/ μ g)
	TA 100	-	1.3-1.9 times the spontaneous reversion frequency (0.04-0.08 rev/ μ g)
	TA 1535	+	1.8 times the spontaneous reversion frequency (0.01 rev/ μ g)
	TA 1537	+	5.9-7.9 times the spontaneous reversion frequency (0.03-0.05 rev/ μ g)
	TA 1538	+	6.0-13.1 times the spontaneous reversion frequency (0.03-0.15 rev/ μ g)
	TA 98	+	4.3-11.1 times the spontaneous reversion frequency (0.03-0.14 rev/ μ g)
Occidental Shale Oil; Residual Oil	TA 100	+	2.2-2.9 times the spontaneous reversion frequency ((0.04-0.12 rev/ μ g)
	TA 1537	-	2.6 times the spontaneous reversion frequency (0.001 rev/ μ g)
	TA 100	-	1.32-1.84 times the spontaneous reversion frequency (0.002-0.009 rev/ μ g)
	TA 1537	+	6.3-9.8 times the spontaneous reversion frequency (0.005-0.007 rev/ μ g)
	TA 1538	+	5.0-10.5 times the spontaneous reversion frequency (0.02-0.03 rev/ μ g)
	TA 98	+	5.9-12.3 times the spontaneous reversion frequency (0.02-0.04 rev/ μ g)
	TA 100	+	2.3-2.6 times the spontaneous reversion frequency (0.01-0.03 rev/ μ g)

Name	Strain	Activation System	Activity
Exxon Donor Solvent; Solvent	TA 1537	+	7.4-11.9 times the spontaneous reversion frequency (0.10-0.20 rev/ μ g)
	TA 1538	+	13.9 times the spontaneous reversion frequency (0.60-1.2 rev/ μ g)
	TA 98	+	16.5-18.5 times the spontaneous reversion frequency (0.82-1.2 rev/ μ g)
	TA 100	+	2.3-2.6 times the spontaneous reversion frequency (0.41-0.44 rev/ μ g)
Exxon Donor Solvent; Vacuum Gas Oil	TA 98	-	2.2 times the spontaneous reversion frequency (0.26 rev/ μ g)
	TA 100	-	2.3 times the spontaneous reversion frequency (1.81 rev/ μ g)
	TA 1535	+	3.4-4.8 times the spontaneous reversion frequency (0.05-0.10 rev/ μ g)
	TA 1537	+	85.2-280.4 times the spontaneous reversion frequency (0.60-0.97 rev/ μ g)
	TA 1538	+	110.2-158.1 times the spontaneous reversion frequency (0.45-5.8 rev/ μ g)
	TA 98	+	75.4-146.0 times the spontaneous reversion frequency (0.63-5.8 rev/ μ g)
	TA 100	+	11.6-28.8 times the spontaneous reversion frequency (1.08-3.5 rev/ μ g)
Exxon Donor Solvent; Heavy Gas Oil	TA 100	-	1.87 times the spontaneous reversion frequency (1.87 rev/ μ g)
	TA 1535	+	2.2-3.2 times the spontaneous reversion frequency (0.04-0.05 rev/ μ g)
	TA 1537	+	36.9-38.9 times the spontaneous reversion frequency (0.38-0.52 rev/ μ g)
	TA 1538	+	53.9-74.2 times the spontaneous reversion frequency (2.2-3.6 rev/ μ g)

Name	Strain	Activation System	Activity
Exxon Donor Solvent; Heavy Gas Oil	TA 98	+	40.7-43.6 times the spontaneous reversion frequency (2.4-3.2 rev/ μ g)
	TA 100	+	5.7-6.6 times the spontaneous reversion frequency (0.9-1.1 rev/ μ g)
Exxon Donor Solvent; Residual Oil	TA 98	-	2.0-2.3 times the spontaneous reversion frequency (1.6-1.8 rev/ μ g)
	TA 100	-	1.3-1.9 times the spontaneous reversion frequency (0.80-0.92 rev/ μ g)
	TA 1535	+	3.7-4.8 times the spontaneous reversion frequency (0.02-0.08 rev/ μ g)
	TA 1537	+	88.1-214.5 times the spontaneous reversion frequency (0.59-0.97 rev/ μ g)
	TA 1538	+	86.8-99.1 times the spontaneous reversion frequency (3.9-5.0 rev/ μ g)
	TA 98	+	60.6-83.2 times the spontaneous reversion frequency (4.5-4.7 rev/ μ g)
	TA 100	+	11.9-14.7 times the spontaneous reversion frequency (0.76-2.6 rev/ μ g)
SRC II; Fuel Oil	TA 1537	+	14.3-22.2 times the spontaneous reversion frequency (0.09-0.17 rev/ μ g)
	TA 1538	+	21.9-22.6 times the spontaneous reversion frequency (0.72-1.5 rev/ μ g)
	TA 98	+	17.4-20.2 times the spontaneous reversion frequency (.98-1.4 rev/ μ g)
	TA 100	+	2.5-2.6 times the spontaneous reversion frequency (0.80-1.06 rev/ μ g)
SRC II; Vacuum Tower Bottoms	TA 1537	-	2.7-4.3 times the spontaneous reversion frequency (0.01-0.02 rev/ μ g)
	TA 1538	-	2.3-3.0 times the spontaneous reversion frequency (0.01 rev/ μ g)

Name	Strain	Activation System	Activity
SRC II; Vacuum Tower Bottoms (contd.)	TA 98	-	2.4-3.0 times the spontaneous reversion frequency (0.02-0.04 rev/ μ g)
	TA 100	-	1.9-2.8 times the spontaneous reversion frequency (0.04-0.16 rev/ μ g)
	TA 1535	+	2.9-3.2 times the spontaneous reversion frequency (0.01-0.02 rev/ μ g)
	TA 1537	+	87.7-88.3 times the spontaneous reversion frequency (0.28-0.43 rev/ μ g)
	TA 1538	+	104.2-114.4 times the spontaneous reversion frequency (0.77-1.8 rev/ μ g)
	TA 98	+	59.8-61.8 times the spontaneous reversion frequency (0.97-1.7 rev/ μ g)
	TA 100	+	9.5-15.0 times the spontaneous reversion frequency (0.88-1.0 rev/ μ g)
SRC II; Stripper Tower Bottoms	TA 1537	-	2.9-4.9 times the spontaneous reversion frequency (0.007-0.02 rev/ μ g)
	TA 1538	-	3.9-4.3 times the spontaneous reversion frequency (0.03-0.05 rev/ μ g)
	TA 98	-	2.2-2.4 times the spontaneous reversion frequency (0.03-0.04 rev/ μ g)
	TA 100	-	1.5-3.3 times the spontaneous reversion frequency (0.24-0.28 rev/ μ g)
	TA 1535	+	2.2-2.5 times the spontaneous reversion frequency (0.04-0.05 rev/ μ g)
	TA 1537	+	50.0-116.6 times the spontaneous reversion frequency (0.42-0.61 rev/ μ g)
	TA 1538	+	91.0-189.9 times the spontaneous reversion frequency (1.3-3.7 rev/ μ g)
	TA 98	+	52.7-102.6 times the spontaneous reversion frequency (1.2-4.6 rev/ μ g)

Name	Strain	Activation System	Activity
SRC II; Vacuum Tower Bottoms (contd.)	TA 100	+	6.4-14.3 times the spontaneous reversion frequency (0.65-1.8 rev/ μ g)
SRC II; Heavy Gas Oil	TA 1535	+	2.0-2.3 times the spontaneous reversion frequency (0.05-0.41 rev/ μ g)
	TA 1537	+	18.0-33.0 times the spontaneous reversion frequency (0.77-0.86 rev/ μ g)
	TA 1538	+	71.4-97.8 times the spontaneous reversion frequency (4.5-6.1 rev/ μ g)
	TA 98	+	49.3-51.7 times the spontaneous reversion frequency (4.7-6.5 rev/ μ g)
	TA 100	+	6.2-8.2 times the spontaneous reversion frequency (2.0-3.3 rev/ μ g)
SRC II; Residual Oil	TA 1537	-	2.7-3.6 times the spontaneous reversion frequency (0.03 rev/ μ g)
	TA 1538	-	2.3 times the spontaneous reversion frequency (0.02-0.03 rev/ μ g)
	TA 98	-	2.6-2.7 times the spontaneous reversion frequency (0.04-0.05 rev/ μ g)
	TA 100	-	1.8-2.8 times the spontaneous reversion frequency (0.16-0.18 rev/ μ g)
	TA 1535	+	2.9-3.5 times the spontaneous reversion frequency (0.05-0.11 rev/ μ g)
	TA 1537	+	97.9-136.7 times the spontaneous reversion frequency (1.1-1.6 rev/ μ g)
	TA 1538	+	143.8-154.7 times the spontaneous reversion frequency (3.7-5.6 rev/ μ g)
	TA 98	+	66.9-108.4 times the spontaneous reversion frequency (3.4-5.5 rev/ μ g)
	TA 100	+	11.8-14.3 times the spontaneous reversion frequency (2.02-3.7 rev/ μ g)

TABLE XV

MOUSE SKIN PAINTING INITIATION-PROMOTION STUDY

Sample	# Mice With Tumors Lasting >30 Days	Average # Tumors/ Tumor Bearing Mouse	Number of Days to 1st Mouse With Tumor
Controls			
Toluene/Acetone	0	0	0
Toluene/PMD	4	1.5	111
None/PMA	3	1.0	92
Benzo(a)pyrene	26	7.4	48
South Louisiana Petroleum			
Naphtha	2	1.0	111
LGO*	3	1.7	71
MGO	3	1.0	83
Residue	5	1.6	62
Crude*	5	1.4	71
H Coal Syncrude Mode			
ASO*	2	2.0	<
Naphtha	0	0	92
LGO	1	2.0	76
ASB*	14	3.4	41
VSO	20	5.2	41
VSB	15	3.1	51
Paraho Shale Oil			
Naphtha	0	0	<
LGO	0	0	<
MGO*	2	1.5	113
Residue*	7	3.1	62
Crude	9	1.9	59
Geokinetics Shale Oil			
Crude	2	1.0	92
Naphtha*	3	1.3	76
LGO	1	1.0	59
MGO	1	1.0	85
Residue+	2	1.5	83

*29 Surviving mice in group

+ Surviving mice in group

TABLE XV (CONTD.)

Sample	# Mice With Tumors Lasting >30 Days	Average # Tumors/ Tumor Bearing Mouse	Number of Days to 1st Mouse With Tumor
Toluene/Acetone	0	0	-
Toluene/PMA	1	2.0	97
None/PMA	1	1.0	105
Benzo(a)pyrene	25	4.5	44
H Coal Fuel Oil Mode			
ASO	2	1.0	56
ASB	14	2.4	44
VSO	24	4.5	37
VSB	13	2.4	37
Occidental Shale Oil			
Naphtha	1	1	135
LGO	1	2.0	84
MGO	4	1.3	98
Crude	0	0	-
Residue	3	1.7	98
Exxon Donor Solvent			
Naphtha	0	0	-
Solvent	12	2.5	63
HGO	18	3.6	42
VGO	20	3.3	51
Residue	17	2.4	42
SRC II			
Naphtha	3	1.0	63
Fuel	9	2.3	51
HGO	18	3.1	42
STB	15	2.5	51
VTB	10	1.9	42
Residue	21	3.0	51

TABLE XVI

COMPARISON OF THE SALMONELLA AND INITIATION-PROMOTION TEST RESULTS

SOUTH LOUISIANA PETROLEUM

Fraction	<u>Salmonella Mutation Assay (with Activation)</u>					<u>Initiation-Promotion Assay</u>		
	Strain					Number Mice With Tumors Lasting >30 Days	Total Tumors Lasting >30 Days	Number of Days to First Mouse With Tumor
	TA 1535	TA 1537	TA 1538	TA 98	TA 100			
Naphtha	-	-	-	-	-	2	2	111
Light Gas Oil	-	-	-	-	-	3	5	71
Mid-Gas Oil	-	0.01* (2.5)+	0.02 (1.9)	0.03 (2.0)	0.09 (1.6)	3	3	83
Resid.	-	-	-	0.02 (2.4)	0.4 (2.0)	5	8	62
Crude	-	-	-	-	0.02 (1.8)	5	7	71
Benzo(a)pyrene	8.3 (5.4)	22.2 (23.8)	27.0 (9.9)	75.8 (18.1)	118.3 (8.1)	26	157	48

- Not mutagenic (only refers to Salmonella data)

* Revertants per μg

+ The maximum number of revertants observed expressed as a multiple of the average solvent control.

TABLE XVII

COMPARISON OF THE SALMONELLA AND INITIATION-PROMOTION TEST RESULTSPARAHO SHALE OILSalmonella Mutation Assay (with Activation)Initiation-Promotion Assay

Fraction	Strain					Number Mice With Tumors Lasting >30 Days	Total Tumors Lasting >30 Days	Number Of Days to First Mouse With Tumor
	TA 1535	TA 1537	TA 1538	TA 98	TA 100			
Naphtha	-	-	-	-	-	0	0	-
Light Gas Oil	-	-	-	-	-	0	0	-
Mid-Gas Oil	-	0.05 ^a (8.1)+	0.20 (6.5)	0.20 (6.7)	0.40 ^b (1.8)	2	3	113
Resid	-	0.4 (16.1)	0.80 (31.3)	0.40 (40.6)	0.40 ^c (4.4)	7	22	62
Crude	-	0.07 (12.0)	0.55 (41.9)	0.70 (29.7)	0.50 (2.8)	9	17	59
Benzo(a)pyrene						26	193	48

- Not mutagenic (only refers to Salmonella data).

* Revertants per μg (average).

+ The maximum number of revertants observed expressed as a multiple of the average solvent control reversion frequency.

a Without activation 0.01 (2.3).

b Without activation 0.06 (1.3).

c Without activation 0.03 (1.4).

TABLE XVIII

COMPARISON OF THE SALMONELLA AND INITIATION-PROMOTION TEST RESULTSGEOKINETICS SHALE OIL (in Situ)

Fraction	<u>Salmonella Mutation Assay (with Activation)</u>					<u>Initiation-Promotion Assay</u>		
	TA 1535	TA 1537	Strain TA 1538	TA 98	TA 100	Number Mice With Tumors Lasting >30 Days	Total Tumors Lasting >30 Days	Number Of Days to First Mouse With Tumor
Naphtha	-	-	-	-	-	3	4	75
Light Gas Oil	-	-	-	-	-	1	1	59
Mid-Gas Oil	-	0.01* ^a (9.0)+	0.08 (11.1)	0.20 ^b (18.7)	0.20 (3.0)	1	1	85
Residue	-	0.06 (7.3)	0.40 (29.8)	0.30 (32.6)	0.30 (4.1)	2	3	83
Crude	-	0.03 (4.3)	0.10 (6.0)	0.10 (5.7)	0.09 (1.7)	2	2	92
Benzo(a)pyrene						26	157	48

- Not mutagenic (only refers to Salmonella data).

* Revertants per μg (average).

+ The maximum number of revertants expressed as a multiple of the average solvent control reversion frequency.

a Without activation 0.003 (2.7).

b Without activation 0.004 (2.5).

TABLE XIX

COMPARISON OF THE SALMONELLA AND INITIATION-PROMOTION TEST RESULTS

OCCIDENTAL SHALE OIL (in Situ)

Fraction	<u>Salmonella Mutation Assay (with Activation)</u>					<u>Initiation-Promotion Assay</u>		
	TA 1535	TA 1537	Strain TA 1538	TA 98	TA 100	Number Mice With Tumors Lasting >30 Days	Total Tumors Lasting >30 Days	Number Of Days to First Mouse With Tumor
Naphtha	-	-	-	-	-	1	0	135
Light Gas Oil	-	-	-	0.04 (2.5)	0.07 (1.5)	1	2.0	84
Mid-Gas Oil	0.01* (1.8)+	0.04 (6.9)	0.09 (9.6)	0.09 ^a (7.7)	0.08 ^b (2.6)	4	1.3	98
Residue	-	0.01 ^c (8.1)	0.03 (7.8)	0.03 (9.1)	0.02 ^d (2.5)	3	1.7	98
Crude	-	0.01 (3.9)	0.04 (6.8)	0.05 (8.2)	0.09 (2.5)	0	0	-

- Not mutagenic (only refers to Salmonella data).

* Revertants per μg (average).

+ The maximum number of revertants observed expressed as a multiple of the average solvent control reversion frequency.

a Without activation 0.02 (2.8).

b Without activation 0.06 (1.6).

c Without activation 0.001 (2.6).

d Without activation 0.006 (1.6).

TABLE XX

COMPARISON OF THE SALMONELLA AND INITIATION-PROMOTION TEST RESULTS

H COAL - SYNCRUDE MODE

Fraction	Salmonella Mutation Assay (with Activation)					Initiation-Promotion Assay		
	TA 1535	TA 1537	Strain TA 1538	TA 98	TA 100	Number With Tumors Lasting >30 Days	Total Tumors Lasting >30 Days	Number Of Days to First Mouse With Tumor
Naphtha	-	-	-	-	-	0	0	-
Light Gas Oil	-	-	-	-	-	1	2	76
Atmos. Still Bottoms (VSB)	-	0.20 (11.5)	1.9 (57.4)	2.8 (38.9)	0.62 (4.0)	14	48	41
Vac. Still Overhead (VSO)	-	0.52 (26.3)	4.5 (87.7)	6.2 (72.5)	1.1 (6.9)	20	103	41
Vacuum Still Bottoms	0.07* (3.8)+	1.4a (379.4)	4.8b (112.5)	4.8c (139.8)	2.7d (19.3)	16	50	61
Atmos. Still Overhead (~Crude)	-	-	-	-	-	2	4	92
Benzo(a)pyrene						26	157	48

- Not mutagenic (only refers to Salmonella data).

* Revertants per μg (average).

+ The maximum number revertants observed expressed as a multiple of the average solvent control reversion frequency.

a Without activation 0.02 (1.8).

b Without activation 0.04 (6.0).

c Without activation 0.07 (4.3).

d Without activation 0.22 (2.2).

TABLE XXI

COMPARISON OF THE SALMONELLA AND INITIATION PROMOTION-TEST RESULTS

H COAL - FUEL OIL MODE

Fraction	Salmonella Mutation Assay (with Activation)					Initiation-Promotion Assay		
	Strain					Number Mice With Tumors Lasting >30 Days	Total Tumors Lasting >30 Days	Number Of Days to First Mouse With Tumor
	TA 1535	TA 1537	TA 1538	TA 98	TA 100			
Atmosphere Still Overhead	-	-	-	-	-	2	1.0	56
Atmosphere Still Bottoms	-	0.14 (13.1)	1.24 (49.6)	1.36 (37.9)	0.45 (3.7)	14	2.4	44
Vacuum Still Overhead	0.02* (2.3)+	0.25 (41.0)	1.6 (66.3)	1.8 (65.7)	1.0 (8.4)	24	4.5	37
Vacuum Still Bottoms	0.03 (2.7)	0.55 (61.8)	2.4 ^a (101.8)	2.3 ^b (79.2)	1.2 ^c (17.4)	13	2.4	37

- Not mutagenic (only refers to Salmonella data).

* Revertants per μg (average).

+ The maximum of revertants observed expressed as a multiple of the average solvent control reversion frequency.

a Without activation 0.003 (2.2).

b Without activation 0.01 (3.2).

c Without activation 0.09 (3.0).

TABLE XXII

COMPARISON OF THE SALMONELLA AND INITIATION-PROMOTION TEST RESULTS

SRC II

Fraction	Salmonella Mutation Assay (with Activation)					Initiation-Promotion Assay		
	TA 1535	TA 1537	Strain TA 1538	TA 98	TA 100	Number Mice With Tumors Lasting >30 Days	Total Tumors Lasting >30 Days	Number Of Days to First Mouse With Tumor
Naphtha	-	-	-	-	-	3	1.0	63
Fuel Oil	-	0.13 (18.3)	1.11 (22.3)	1.16 (18.8)	0.93 (2.6)	9	2.3	51
Heavy Gas Oil	0.23* (2.2)+	0.82 (25.5)	5.3 (84.6)	5.6 (50.5)	2.7 (7.2)	18	3.1	42
Stripper Tower Bottoms	0.05 (2.4)	0.52 ^a (83.3)	2.5 ^b (140.5)	2.9 ^c (77.7)	1.23 ^d (10.4)	15	2.5	51
Vacuum Tower Bottoms	0.02 (3.1)	0.36 ^e (88.0)	1.3 ^f (109.3)	1.34 ^g (60.8)	0.94 ^h (12.3)	10	1.9	63
Residue	0.08 (3.2)	1.4 ⁱ (117.3)	4.7 ^j (149.3)	4.5 ^k (87.7)	2.9 ^l (13.1)	21	3.0	51

- Not mutagenic (only refers to Salmonella data).

* Revertants per μg (average).

+ The maximum number of revertants observed expressed as a multiple of the average solvent control reversion frequency.

a Without activation 0.01 (3.9).

b Without activation 0.04 (4.1).

c Without activation 0.04 (2.3).

d Without activation 0.26 (2.4).

e Without activation 0.02 (3.5).

f Without activation 0.01 (2.7).

g Without activation 0.03 (2.7).

h Without activation 0.10 (2.4).

i Without activation 0.03 (3.2).

j Without activation 0.03 (2.3).

k Without activation 0.05 (2.7).

l Without activation 0.17 (2.3).

TABLE XXIII

COMPARISON OF THE SALMONELLA AND INITIATION-PROMOTION TEST RESULTS

EXXON DONOR SOLVENT PROCESS (COAL)

Fraction	Salmonella Mutation Assay (with Activation)					Initiation-Promotion Assay		
	Strain					Number Mice With Tumors Lasting > 30 Days	Total Tumors Lasting > 30 Days	Number Of Days to First Mouse With Tumor
	TA 1535	TA 1537	TA 1538	TA 98	TA 100			
Naphtha	-	-	-	-	-	0	0	-
Solvent	-	0.15 (9.7)	0.90 (13.9)	1.01 (17.5)	0.43 (2.45)	12	2.5	63
Heavy Gas Oil	0.05* (2.7)+	0.45 (37.9)	2.9 (64.1)	2.8 (42.2)	1.0 ^a (6.2)	18	3.6	42
Vacuum Gas Oil	0.08 (4.1)	0.79 (182.8)	3.13 (134.2)	3.22 ^b (110.7)	2.18 ^c (20.2)	20	3.3	51
Residual Oil	0.05 (4.3)	0.78 (151.3)	4.45 (93.0)	4.6 ^d (71.9)	1.68 ^e (13.3)	17	2.4	42

- Not mutagenic (only refers to Salmonella data).

* Revertants per μg (average).

+ The maximum number of revertants observed as a multiple of the average solvent control reversion frequency.

a Without activation 1.87 (1.87).

b Without activation 0.26 (2.2).

c Without activation 1.81 (2.3).

d Without activation 1.7 (2.2).

e Without activation 0.86 (1.6).

APPENDIX I

Identification of Samples Tested

1) The South Louisiana Crude Petroleum control was an American Petroleum Institute (API) Reference Sample obtained from Professor Jerry M. Neff of Texas A&M University. It was reported to have the following analysis.

API Gravity @ 60°F	34.5
Sulfur (wt %)	.25
Nitrogen (wt %)	.069
Nickel (ppm)	2.2
Vanadium (ppm)	1.9
Naphtha (up to 400°F)wt%	18.6
Saturates (wt %)	56.3
Aromatics (wt %)	16.5
Polars (wt%)	8.4
Insolubles	0.2

2) H Coal syncrude mode (Hydrocarbon Research, Inc.)

This sample was produced from Illinois No. 6 coal (from the Burning Star Mine) in the syncrude mode of operation from PDU Run 5 in September, 1977.

3) H Coal fuel oil mode (Hydrocarbon Research, Inc.)

This sample was produced from Illinois No. 6 coal in the fuel oil mode from PDU Run 3 during April and May, 1976.

4) SRC II (The Pittsburgh & Midway Coal Mining Co.)

This was produced from Pittsburgh 8 seam coal from the North American Coal Corp. Powhattan No. 5 Mine (Powhattan Point, Belmont County, Ohio) by the SRC recycle slurry mode of operation during October, 1978 to February, 1979.

5) Exxon Donor Solvent Coal Liquid (Exxon Research and Engineering Co.)

This material was produced in the Exxon 1 ton per day liquefaction unit in September, 1977 from Illinois #6 coal from the Monterey Mine.

Conditions: temperature	840°F
pressure	1500 psi
Normal residence time	40 min.
Solvent to coal ratio	1.2

6) Paraho Shale Oil (Development Engineering Inc.)

This material was produced in the Paraho direct heated retort and is representative of the large 100,000 barrels of oil produced in 1978 for large scale refining and military use tests. Its general properties were reported as:

APPENDIX I (CONTD.)

API Gravity	21.6
Sediment	.68
Water Vol. %	.02
Sulfur wt %	.64
Nitrogen wt %	2.04
Pour Point °F	85

7) Occidental In-Situ Shale Oil (Occidental Petroleum Co.)
This material was produced by Occidental's In Situ Shale Retorting process during the tests of Retort #6 in fall, 1978 and winter, 1979.

8) Geokinetics In situ Shale Oil (Geokinetics, Inc.)
This material was produced by the Geokinetics in situ Shale oil process; presumably in 1978.

APPENDIX II

Distillation Procedures

Syncrude samples for this study were separated into four distillation fractions, the distillation procedure following ASTM D2892-73(3) modified to minimize contamination or thermal degradation. The distillation equipment was a one inch diameter, 27 inch high, 20 plate Oldershaw column with 3 liter still pot. The glass vacuum jacket was heated with external heating tapes to minimize heat loss from the column. Pipe flanges with Teflon® gaskets eliminated ground joints and grease which could reflux back to the still pot. A magnetic stirrer with Teflon® coated stirring bar and "Hengar" stone boiling chips reduced bumping within the pot and overheating. Heat to the still pot was supplied by a "Variac". A gooseneck was installed above the receiver to reduce mechanical shocks by allowing the entire still to move slightly if bumping occurred in the pot (4).

Details of running the distillation are not significantly different from ASTM 2892. Control of the temperatures and boil-up rates was by hand setting of Variacs using thermocouples attached to a multipoint recorder. The distillation cuts were taken at points specified in Table A-I. Accurate vacuum control was maintained by balancing a nitrogen bleed and a vacuum source with a Moore pressure regulator.

Water in the crude samples was sometimes a problem. In addition to the "bumping" of the pot liquids as the water phase flashes, the steam caused some steam distillation to occur. For samples with much water, the organic phase distillate was decanted and returned to the pot and the distillation resumed (5).

Prior to all runs, the still was cleaned with Isopropanol and methylene chloride and then flushed with acetone and blown dry. All tubing was changed and the pressure gauges checked against manometers.

TABLE A-I

Desired Laboratory Distillation Fractions*

Observed Temperature at Reduced Pressure

	<u>Pressure</u> mm Hg	<u>Initial B.P.</u>	<u>Final B.P.</u>	<u>Expected</u> <u>Max. Pot Temp.</u>
Light Ends	700	Trap Liquids Returned to Naphtha		
Naphtha <350°C	700	50°C	175°C	275°C
LGO Light Gas Oil Mid Distillate (350 to 650°F)	5	50°C	179°C	279°C
MGO Mid Gas Oil (650 to 850°F)	1	145°C	210°	260 to 290°C
Resid		Pot Residue Pours at 50 to 100°C		

*Temperatures for end points were adjusted using the correlation of Table X, ASTM Std. D2892 and the pressure actually achieved. Table III shows results actually achieved.

Figure 1 -
20 Plate
Oldershaw
Distillation
Column

Figure 2 - Distillation Column
Controls

APPENDIX III

Analytical Testing of Crude Samples and Distillation Cuts

It is recognized that complete characterization (quantitative and qualitative analysis of the mixture constituents) of crude samples and their distillation cuts is impractical both technically and economically. Measurement of mutagenicity of specific compounds or specific groups of compounds is outside the scope of this study.

Some sample characterization is desirable, however, to insure that the samples studied are typical.

a) Tests Selected

Elemental analyses were selected as the most reasonable measure of the crude and fractions gross composition.

The ASTM Simulated Distillation Analysis (Boiling Range Distribution of Petroleum Fractions by Gas Chromatography) was chosen because it represents a means of monitoring the proper execution of the distillation of the crude samples and the fractions.

Specific Gravity is a well recognized standard measurement in most petroleum laboratories and characterizes the sample in terms understood in the industry.

b) Facilities

All analytical testing was conducted in facilities of the E. I. du Pont de Nemours & Company, Inc., located at the Experimental Station, Wilmington, Delaware.

c) Tests Used

Carbon-Hydrogen Analysis

Apparatus: Coleman Carbon-Hydrogen Analyzer (or equivalent).

Technique: Combustion analysis followed by gravimetric determination of CO_2 and H_2O .

Nitrogen Analysis

Apparatus: Nitrogen-Dumas

Technique: Combustion analysis followed by volumetric determination of N_2 .

Alternate equipment Perkin Elmer Auto Analyzer or Micro Kjeldahl apparatus.

APPENDIX III CONTINUED

Oxygen Analysis

Apparatus: Laboratory Equipment Co. (LECO) Nitrox-6 Analyzer.

Technique: Combustion analysis - followed by gas chromatographic determination of CO.

Sulfur Analysis

Apparatus: Philips Universal Vacuum Spectrometer (X-ray Fluorescence) equipped with:

1. An unfiltered chromium target tube operated at 50 KV and 35 ma.
2. A PET (pentaerythritol) analyzing crystal.
3. A gas flow proportional counter with a gas density compensator controlling the flow of P-10 gas to the detector.
4. A helium gas system to sweep the entire spectrometer chamber.

Technique: K-emissions from the sulfur in the sample are excited by radiation from a chromium target x-ray tube. K intensities in terms of concentration are measured, using a series of NBS standards of sulfur in residual fuel oil.

Gravimetric Sulfur

Sample is combusted in Parr-bomb precipitated as barium salt, separated, dried and weighed.

LECO Sulfur Analyzer

Sample is combusted at 2500°F in a stream of oxygen. Infrared absorption of SO₂ evolved is measured and compared with a sulfur standard.

The LECO-sulfur Determination yielded a combustion residue for 7 of these samples which was weighed. This residue is reported in the analysis tables as ash at 2500°F.

Density

Seven samples were viscous tars or solids at room temperature. The density of these was measured using a helium pycnometer. Helium displacement is used in this method to determine the sample volume. The instrument is calibrated at 22°C, thus these determinations were made at 22°C.

APPENDIX III (CONTD.)

Specific Gravity

Apparatus: Pycnometers, etc. as required.

Technique: Defined in ASTM method D-1217-54
D-1480-62

Water displacement in Hubbard-Carmick pycnometer.

Simulated Distillation Analysis

Apparatus: A Hewlett Packard 5730A gas chromatograph equipped with thermal conductivity detector. Data is collected and handled by the Du Pont PDP 10 GC Data Analysis System which includes some refinements not available in the ASTM method.

Technique: ASTM method D-2887-73 - Standard Method of Test for Boiling Range Distributions of Petroleum Fractions by Gas Chromatography.

APPENDIX IV

BIOLOGICAL TESTS

A. Mutagenicity evaluation with *Salmonella typhimurium*.

Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 were treated in the presence and absence of an activation system by a method similar to that described by Ames et al. (*). Treatments without activation were conducted by adding 0.1 ml of the solvent or a solution of the test sample and 0.1 ml of an overnight culture containing approximately 10^8 bacteria to 2 ml of top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). These components were mixed and poured on the surface of a plate containing 20 ml of Davis minimal agar. Treatments with activation were conducted by adding 0.5 ml of S-9 mix to the bacteria/test sample/top agar and pouring the mixture onto a minimal agar plate. The S-9 mix contained per ml: 0.3 ml of S-9, 8 micromoles $MgCl_2$, 33 micromoles KCl, 5 micromoles glucose-6phosphate, 4 micromoles NADP and 100 micromoles sodium phosphate (from a 0.2 M stock sodium phosphate solution at pH 7.4). The S-9 was the 9,000 x g supernatant of liver homogenate (1 gm wet liver:3 ml 0.15 M KCl) from 8 to 9 week old male Charles River CD rats given 500 mg Aroclor 1254/kg five days before sacrifice. The revertant colonies were counted after the plates were incubated at 37°C for 48 hours.

Positive controls were included in each experiment. They were: N-methyl-N'-nitro-N-nitrosoguanidine (TA 1535 and TA 100 without activation), 9-aminoacridine (TA 1537 without activation), 2-nitrofluorene (TA 1538 and TA 98 without activation) and 2-aminoanthracene (all strains with activation). The solvent for a sample was dimethylsulfoxide (DMSO). It was necessary to heat and stir some fractions to maximize solubility. Nontoxic and, if possible, slightly toxic concentrations of each test sample were selected for the mutation experiments after conducting preliminary toxicity studies with strain TA 1535 in plates containing excess histidine.

Each test sample was tested at five or more concentrations and duplicate plates were treated for each point. All experiments were duplicated.

The data were analyzed statistically. The Z-test was used to determine whether the revertant numbers at each concentration of test chemical were different from control values and the Spearman rank correlation was used to determine if there was a linear dose-response. A test sample was classified as mutagenic if both a significant increase in total revertant colony number ($p \leq 0.01$) and a dose-response ($p \leq 0.05$) were observed.

* Ames, B. N., McCann, J. and Yamasaki, E. (1975). Methods for Detecting Carcinogens and Mutagens with the *Salmonella* Mammalian Microsome Mutagenicity Test. Mutation Res. 31:347-364.

APPENDIX IV (CONTINUED)

Solvent controls (dimethylsulfoxide) were run with each test sample. The average solvent control value and standard derivation for each condition were: TA 1535 without activation (23.4 ± 7.1), with activation (21.6 ± 6.0); TA 1537 without activation (9.5 ± 3.7), with activation (9.4 ± 3.6); TA 1538 without activation (14.0 ± 4.8), with activation (27.2 ± 7.3); TA 98 without activation (21.2 ± 5.6), with activation (35.0 ± 9.0); TA 100 without activation (127.5 ± 21.3), with activation (158.4 ± 23.9).

APPENDIX IV. (CONTINUED)

B. Mouse-Skin Painting Initiation-Promotion Test

Animals Swiss ICR/Ha, females, 6 weeks old, 30 mice/test group, housed 2 per cage, water and lab chow ad lib, one square inch patch on back shaved and kept free of hair throughout the experiment.

Exposure Schedule Administer to shaved area approximately 0.1 ml test material as received or as a toluene paste at start of experiment. Two weeks later begin painting 2.5 µg phorbol for 180 days. Positive control benzo(a)pyrene will be administered in a like manner. There are two negative controls: the PMA promoter in acetone and the acetone control will be administered on each treatment day to mice which receive toluene at initiation.

Duration Animals will be sacrificed when moribund or when they develop highly advanced skin tumors. Otherwise, the promoter treatment will continue for 180 days.

Parameters to be measured:

1. Body weight recorded weekly
2. Time to tumor(s) recorded as tumors occur
3. Tumors arising in the skin will be charted monthly
Tumors must persist for 30 days to be counted.
4. Number of tumors per animal
5. Mortality