SYNTHETIC CRUDE OILS CARCINOGENICITY SCREENING TESTS

PROGRESS REPORT

Covering the Period February 15, 1979 to September 15, 1979

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ABSTRACT

Four crude oils (Southern Louisiana Crude Petroleum, H Coal Syncrude, Paraho Crude Shale Oil and Geokinetics in situ Shale Oil) which were distilled into four fractions (naphtha, mid-distillate, gas oil and residue) for analysis and biological screening testing during the first report period were tested for mutagenicity by the Ames test and for tumor initiating activity by an initiation/promotion (skin painting) test. Substantial agreement exists between Ames and skin painting results.

Low boiling fractions of the 4 crude oils showed little or no mutagenicity or tumor activity by the two tests used. The higher boiling fractions (mid gas oils and residues) and the crude oils themselves showed positive mutagenicity and tumor initiation activity. The coal derived fractions were more potent by both tests while the shale oil fractions showed greater activity than the petroleum fractions but considerably less than the coal syncrude.

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Syncrude Carcinogenicity Screening Tests
Quarterly Technical Progress Report for the Period
February 15 - September 15

INTRODUCTION

This Report is the second Technical Progress Report for the U.S. Department of Energy Agreement No. EP-78-C-02-4758.

The objective of this project is to define in a preliminary fashion the carcinogenicity potential that exists in the production, handling and utilization of synthetic crude oils and distillation fractions derived from them. The study involves conducting a rapid screening series of mutagenicity and tumor initiation promotion tests on original crude oils and common distillation fractions of a crude petroleum control and seven synthetic crude oils derived from coal or oil shale by several processes currently under development. Selected analytical tests are run on the fractions to provide some characterization of the samples. The biological tests [Ames test (1) and mouse skin initiation-promotion test (2)] have been extensively studied and are designed to provide on a short term basis an indication of which materials are potentially more hazardous and should be investigated more thoroughly by longer term testing procedures.

The project is being run in two parts; in each part, processing four crude oils. The first four samples (S. Louisiana crude petroleum, H coal Syncrude, Paraho Shale Oil and Geokinetics in situ Shale Oil) were distilled and analyzed in the previous report period and the analytical results were reported in the first progress report. The biological test results are reported herein.

The second part of this project covers the processing, analyzing and testing of four additional synthetic crude oils. They are Exxon Donor Solvent Liquid, SRC-II, H Coal in the fuel oil mode, and Occidental in-situ shale oil. The fractionation and analysis of these materials are included in this report. The biological tests, however, are just getting underway and will only be mentioned here.

I. Phase I

A. Biological Testing of the First Crude Oil Series (South Louisiana Crude Petroleum, H Coal Syncrude, Paraho Shale Oil, and Geokinetics in situ Shale Oil)

Two types of accelerated biological tests are being used to screen these synthetic crude oils and their distillation fractions:

- Ames Salmonella/microsome assay
- Mouse skin initiation/promotion test

Tests using these methods have been completed on the crude oils processed under Phase I. All of these samples were separated into four fractions by distillation as described in Progress Report I. The biological test results are reported below.

B. Ames Tests (7) (8) (9) (10)

Ames Tests on crude oils have been done according to the standard plate incorporation assay using DMSO* as solvent (1). The ability of each test sample to revert Salmonella typhimurium strains TA 1535, 1537, 1538, 98 and 100 from histidine dependence to histidine independence was determined. Details of the conduct and interpretation of the test are provided in Appendix I. Both positive (selected mutagens) and negative controls were included and tests were run with and without activation (a rat liver homogenate was used). Cytotoxicity of each sample was determined in the presence and absence of the activation system to provide the basis for selecting concentrations to be used in the mutagenesis experiments.

Dramatic differences in the solubilities of test samples in both DMSO and the aqueous agar test medium were observed. Stirring and heating of DMSO-test sample mixtures were used to obtain solutions or emulsions that could be accurately aliquotted to the test medium. The mid gas oil, crude, and residue samples tended to pose the greatest solubility difficulties resulting in the least homogeneous test mixtures. Consequently, test concentrations listed for these fractions should be considered approximate. However, given this limitation, the ability of the assay to detect mutagenic activity among these fractions has been clearly demonstrated.

Based on the tests and the criteria established for mutagenesis (Appendix I), the crude oils and fractions tested are rated as shown in Table I. Experimental data upon which these

assignments are based are shown in Appendices II and III. It can be seen that, as might be expected, the low boiling fractions (naphtha and light gas oils) of all four crude oils tested were found to be nonmutagenic. All the higher boiling fractions (mid gas oil and residue) and the crude oils themselves showed some degree of mutagenicity. While the high boiling materials of all the crude oils tested are mutagenic, they clearly differ among themselves in potency. The petroleum crude and higher fractions are very slightly mutagenic and the H coal vacuum still overheads are strongly mutagenic. The higher boiling shale oil fractions and crude oils were in between these two types of materials in terms of potency.

It is interesting to note that the strains of Salmonella showing the most activity with the crude oils and fractions under study were TA 98, TA 1537, and TA 1538 which are frame shift mutants. This observation is consistent with the postulated frame shift activity of the polynuclear aromatic hydrocarbons, phenols and aromatic amine compounds which are believed to be constituents of these crude oils. Some samples were active in Strain TA 100, a base pair substitution mutant.

C. Initiation-Promotion Mouse Skin Painting Tests (2)(3)(4)
(5)(6)

Details of the test procedure for the initiation-promotion mouse skin painting test are given in Appendix IV. The procedure is started by the application of approximately 2 mg of the sample either as is or in 0.05 ml toluene to one square inch shaved patches on the backs of 30 Swiss ICR/HA female 6-week old mice. Two weeks later, application of phorbol myristate acetate in acetone is started on a three times per week basis for six months. Appendix V shows the manner in which the sample was applied in each experiment.

A benz(a) pyrene positive control was run and also two negative controls (toluene/acetone and toluene/PMA). Table II shows the results obtained for the various Phase I crude oils and fractions. Examination of this Table shows very slight tumor initiating activity for the lower boiling fractions (naphthas and light gas oils) of the four crude oils tested. However, the high boiling fractions (medium gas oils and resids) and the crude oils themselves were more active. Of the active high boiling fractions and crude oils, the coal derived H coal materials were the most potent, particularly the vacuum still overhead fraction which approached the benz(a) pyrene control in activity. The shale oil crudes and high boiling fractions were similar in tumor initiating activity to the petroleum crude and high boiling fractions. The Paraho samples were a little more potent and the Geokinetics samples a little less potent than the South Louisiana petroleum control.

TABLE I

SUMMARY: Ames Test Results for Phase I Samples

South Louisiana Crude Petroleum

naphtha	nonmutagenic
light gas oil	nonmutagenic
mid-gas oil	mutagenic
residue	mutagenic
crude oil	mutagenic

H Coal Liquid Syncrude Mode

naphtha	nonmutagenic
light gas oil	nonmutagenic
atmospheric still overheads	nonmutagenic
atmospheric still bottoms	mutagenic
vacuum still overheads	mutagenic

Paraho Shale Oil

naphtha	nonmutagenic
light gas oil	nonmutagenic
mid-gas oil	mutagenic
residue	mutagenic
crude oil	mutagenic

Geokinetics In Situ Shale Oil

naphtha	nonmutagenic
light gas oil	nonmutagenic
mid-gas oil	mutagenic
residue	mutagenic
crude oil	mutagenic

TABLE II

MOUSE SKIN PAINTING INITIATION/PROMOTION STUDY

Date: 7/2/79

Day of Promotion: 180

Group	Sample	# Mice with Tumors lasting >30 days	Total # tumors lasting >30 days	Average # Tumors/ Tumor bearing Mouse	Number of Days to 1st Mouse with Tumor
I	Toluene/Acetone	0	0	0	0
II	Toluene/PMD	4	6	1.5	111
III	None/PMA	3	3	1.0	92
IV	benzo(a)pyrene (H-11,269)	26	193	7.4	48
V	S. Louisiana Naphtha (H-12,760)	2	2	1.0	111
VI	S. Louisiana LGO (H-12,761)*	3	5	1.7	71
VII	S. Louisiana MGO (H-12,762)	3	3	1.0	83
VIII	S. Louisiana Residue (H-12,763)	5	8	1.6	62
IX	S. Louisiana Crude (H-12,764)*	5	7	1.4	71
X	H-Coal ASO (H-12,765)*	2	4	2.0	92
XI	H-Coal Naphtha (H-12,766)	0	0	0	--
XII	H-Coal LGO (H-12,767)	1	2	2.0	76
XIII	H-Coal ASB (H-12,768)*	14	48	3.4	41
XIV	H-Coal VSO (H-12,769)	20	103	5.2	41
XV	Paraho Naphtha (H-12,770)	0	0	0	--
XVI	Paraho LGO (H-12,771)	0	0	0	--
XVII	Paraho MGO (H-12,772)*	2	3	1.5	113
XVIII	Paraho Residue (H-12,773)*	7	22	3.1	62
XIX	Paraho Crude (H-12,774)	9	17	1.9	59
XX	Geokinetics Crude (H-12, 775)	2	2	1.0	92
XXI	Geokinetics Naphtha (H-12,776)*	3	4	1.3	76
XXII	Geokinetics LGO (H-12,777)	1	1	1.0	59
XXIII	Geokinetics MGO (H-12,778)	1	1	1.0	85
XXIV	Geokinetics Residue (H-12,779)+	2	3	1.5	83

*29 surviving mice in group

+28 surviving mice in group

D. Correlation of Salmonella (in vitro) and Initiation/
Promotion (in vivo) Tests

Since two independent tests were selected for screening these crude oils and fractions, it is of interest to compare the results obtained thus far by the two methods. Tables III through VI show these comparative results. In most cases, the qualitative agreement between the two tests is good. Less satisfactory agreement was obtained between the two methods for the Geokinetics samples. Since the Ames tests on the two shale oil types were quite similar, it would appear that the initiation/promotion test on the Geokinetics sample showed lower than expected tumor initiation activity. The reason for this is not known.

TABLE III

Comparison of In Vitro and In Vivo TestsParaho Shale Oil

	<u>AMES TEST (in vitro)</u>				<u>INITIATION/PROMOTION TEST (in vivo)</u>		
	<u>STRAIN (with activation)</u>				<u>mice w/tumors lasting >30 days</u>	<u>total tumors lasting >30 days</u>	<u># of days to first tumor</u>
	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>			
Naphtha	-	-	-	-	0	0	-
Light Gas Oil	-	-	-	-	0	0	-
Mid-Gas Oil	0.044* (8.1)†	0.16 (6.5)	0.19 (6.7)	0.38 (1.8)	2	3	113
Resid	0.044 (16.1)	0.80 (31.3)	0.41 (40.6)	0.38 (4.4)	7	22	62
Crude	0.065 (12.0)	0.55 (41.9)	0.64 (29.7)	0.48 (2.8)	9	17	59
Benzo(a)pyrene	TO BE RUN				26	193	48

* Revertants per ug (average). Comparisons of revertant(s) per ug values among strains can be misleading because these values are strongly influenced by spontaneous reversion frequencies.

† The maximum average number of revertants observed expressed as a multiple of the average spontaneous reversion frequency.

TABLE IV

Comparison of In Vitro and In Vivo TestsGeokinetics Shale Oil (in Situ)

	<u>AMES TEST (in vitro)</u>				<u>INITIATION/PROMOTION TEST (in vivo)</u>		
	<u>STRAIN (with activation)</u>				<u>mice w/tumors lasting >30 days</u>	<u>total tumors lasting >30 days</u>	<u># of days to first tumor</u>
	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>			
Naphtha	-	-	-	-	3	4	76
Light Gas Oil	-	-	-	-	1	1	59
Mid-Gas Oil	0.013* (9.0)†	0.083 (11.1)	0.17 (18.7)	0.17 (3.0)	1	1	85
Residue	0.055 (7.3)	0.41 (29.8)	0.30 (32.6)	0.30 (4.1)	2	3	83
Crude	0.025 (4.3)	0.13 (6.0)	0.10 (5.7)	0.083 (1.7)	2	2	92
Benzo(a)pyrene	TO BE RUN				26	157	48

* Revertants per ug (average). Comparisons of revertant(s) per ug values among strains can be misleading because these values are strongly influenced by spontaneous reversion frequencies.

† The maximum average number of revertants observed expressed as a multiple of the average spontaneous reversion frequency.

TABLE V

Comparison of In Vitro and In Vivo TestsSouth Louisiana Petroleum

	<u>AMES TEST (in vitro)</u>				<u>INITIATION/PROMOTION TEST (in vivo)</u>		
	<u>STRAIN (with activation)</u>				<u>mice w/tumors lasting >30 days</u>	<u>total tumors lasting >30 days</u>	<u># of days to first tumor</u>
	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>			
Naphtha	-	-	-	-	2	2	111
Light Gas Oil	-	-	-	-	3	5	71
Mid-Gas Oil	0.011* (2.5)†	0.024 (1.9)	0.031 (2.0)	0.084 (1.6)	3	3	83
Resid	-	-	0.015 (2.3)	0.035 (1.9)	5	8	62
Crude	-	-	-	0.026 (1.8)	5	7	71
Benzo(a)pyrene	TO BE RUN				26	157	48

* Revertants per ug (average). Comparisons of revertant(s) per ug values among strains can be misleading because these values are strongly influenced by spontaneous reversion frequencies.

† The maximum average number of revertants observed expressed as a multiple of the average spontaneous reversion frequency.

TABLE VI

Comparison of In Vitro and In Vivo TestsH Coal - Syncrude Mode

	<u>AMES TEST (in vitro)</u>				<u>INITIATION/PROMOTION TEST (in vivo)</u>		
	<u>STRAIN (with activation)</u>				<u>mice w/tumors lasting 30 days</u>	<u>total tumors lasting 30 days</u>	<u># of days to first tumor</u>
	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>			
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
Atmos. Still Overhead (~Crude)	-	-	-	-	2	4	92
Benzo(a)pyrene	TO BE RUN				26	157	48

* Revertants per ug (average). Comparisons of revertant(s) per ug values among strains can be misleading because these values are strongly influenced by spontaneous frequencies.

† The maximum average number of revertants observed expressed as a multiple of the average spontaneous reversion frequency.

II. Phase II: Preparation of Samples for the Second Crude Oil Series (EDS, SRC-II, H Coal Fuel Oil and Occidental in situ Shale Oil).

A. Sample Preparation by Distillation

The second set of four samples was prepared in a manner similar to the method reported in the first progress report. The Occidental whole crude sample was distilled in the same equipment described previously. The other three samples were received as separate cuts from the suppliers. In addition to testing the "as received" samples, the vacuum gas oil boiling range material was distilled into two fractions.

The equipment for distilling these vacuum gas oils was like that previously described; however, the Oldershaw column was replaced with a 10 inch long, 1-1/2 inch diameter Vigreux column made from glass pipe. The column pressure drop was then sufficiently low to allow the gas oil to distill overhead.

The procedure for distilling within the Vigreux column is like that described previously. The goal was to distill one fourth to one-half the vacuum gas oil overhead. Distillation was to be stopped if the pot exceeded 325°C or the material began to thermally crack. The cracking is noted if the head temperature drops or vacuum cannot be maintained.

B. Distillation of Second Four Samples

The second four crude oil samples have been prepared as shown in Tables VII and VIII. The samples were nitrogen blanketed and sealed in glass ampoules (or vials if the material was too thick to be put in ampoules). Because of the different forms in which the crude oils were received, there were some differences in the way the distillations were handled:

1. Occidental Shale Oil contained water which distilled with the naphtha. To remove the cloudy water droplets from the organic, a small volume of salt water was shaken with the naphtha and separated by gravity. This first naphtha was returned to the pot and the distillation restarted.

2. Exxon Donor Solvent naphtha and "solvent" were used as received but the Vacuum Gas Oil was further distilled. The distillate obtained was labeled as Heavy Gas Oil (HGO) and the pot residue was labeled "resid". This HGO was so viscous that poor mixing was achieved in the receiver. Only A Vigreux Column was used for the distillation.
3. The SRC-II naphtha, fuel oil and vacuum tower bottoms were used "as is", but the SRC-II stripper tower bottom was distilled into heavy gas oil and resid using a Vigreux Column.
4. H Coal-Fuel Oil mode samples were not distilled but are being tested as received. In addition, the H Coal-Syncrude mode, Vacuum Gas Oil from the earlier set was submitted for testing.

C. Analytical Testing of Crude Samples and Distillation Cuts

Analytical sample characterization tests which were run on the crude samples and distillation cuts studied in the first phase of this work were also run on all samples in the second phase. A description of the tests, the equipment and the facilities used was given in the first progress report of 10/16/78 to 2/15/79. The tests are:

- Elemental analysis for

Carbon
Hydrogen
Nitrogen
Oxygen
Sulfur

- Simulated distillation analysis
- Density

No changes in any of the tests or apparatus used were made except as noted below.

Sulfur

Eight sulfur analyses were not amenable to x-ray fluorescence analysis. These samples were analyzed for sulfur by two different techniques.

- Gravimetric

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 were 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.

Analytical Results

The analytical results of tests conducted on the four syncrudes and their distillation cuts or process products are given in Tables IX to XII.

Biological Testing

Ames tests and initiation/promotion tests on Phase II samples are now being started using the same procedures as were used in Phase I. Results of these tests will be reported in the next progress report. One observation, however, can be made at this time. The SRC-II naphtha sample, unlike the other naphtha samples run in this test program showed some degree of acute toxicity through skin absorption at a fairly high dose level (1.6 gms per kilo of body weight). Of the 30 mice painted with this material, 23 died within 48 hours. A repeat of these tests with 10 additional mice under conditions where inhalation of the fumes from the sample was minimized, still resulted in death of 7 mice. After investigation of the effect of reducing sample size by dilution with toluene, the initiation/promotion testing of all Phase II test samples is proceeding.

TABLE VII

DOE - MUTAGEN STUDY SAMPLES - DISTILLATION CUTS

Order of Distillation	1	2	3	Not Distilled
Sample of	Occidental Shale Oil	Exxon Donor Solvent	SRC-II	H-Coal
Still Feed	Whole Crude	Vacuum Gas Oil	Stripper Tower Bottoms	Not Distilled
Cuts Obtained, Wt.% of Feed				
Light Ends	0	0	0	-
Naptha	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	-
Feed Received in:	5 Gal. Lacquer Can	1 Gal. Glass Jugs	5 Gal. Oil Cans	Lacquer Cans
Samples Forwarded for Testing	Crude* Naptha* LGO MGO* Resid*	Naptha Solvent Vacuum Gas Oil* HGO* Resid*	Naptha 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

TABLE VIII

OBSERVED CUT POINTS - DOE MUTAGEN STUDY SAMPLES*

	<u>Occidental Shale Oil</u>	<u>EDS</u>	<u>SRC-II</u>
<u>Light Ends</u> - Collected in dry ice/acetone trap while naptha distilled			
<u>Naptha</u> (IBP to 350°F equivalence)			
Pressure, Mn-Hg	100	-	-
Temp. °C	108		
<u>LGO</u> (350 to 650°F equivalence)			
Pressure, Mn-Hg	5.0	-	-
Temp. °C	180		
<u>MGO</u> (650-850°F equivalence)			
Pressure, Mn-Hg	1.8	-	-
Temp. °C	193		
<u>HGO</u> (IBP to 950°C equivalence)			
Pressure, Mn-Hg	-	2.2	1.9
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

*H Coal not distilled

TABLE IX

ANALYTICAL RESULTS
H-COAL-FUEL OIL MODE

<u>Sample Description</u>	<u>ASOH</u>	<u>ASB</u>	<u>VSOH</u>	<u>VSB</u>	<u>VSB</u> ⁽¹⁾
<u>Notebook #16928</u>	<u>90-1 to 10</u>	<u>90-11 to 20</u>	<u>90-21 to 30</u>	<u>91-1 to 10</u>	<u>97-1 to 10</u>
<u>Elemental Analysis</u>					
C	85.58	88.58	88.88	70.89	66.24
H	12.02	9.01	8.47	4.56	4.16
N	.86	.78	.69	1.28	1.25
O	1.45	1.54	1.40	10.84	12.91
S	.41	.12	.15	1.81	2.23
Total	100.32	100.03	99.59	89.38	86.79
% Ash @ 2500°F				17.57	23.78
<u>Simulated Distillation Analysis (°C)</u>					
% Light Ends (0-65)	2	0	0	0	0
% Naphtha (65-175)	50	1	1	0	0
% Lt. Gas Oil (175-340)	49	74	51	33	33
% Med. Gas Oil (340-430)	0	24	38	35	34
% Heavy Gas Oil (430-500)	0	2	10	20	18
% Unvolatilized Sample, 300°C	2.17	<2.00	4.24	~58.2	~89.4
<u>Density</u> (25°C) g/cc	.8442	.9926	1.031	1.49*	1.41*

*Helium Pycnometer at 22°C

(1) VSB from H-Coal-Syncrude Mode

TABLE X

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>Notebook #16928</u>	<u>95-1 to 10</u>	<u>95-11 to 20</u>	<u>99-1 to 10</u>	<u>99-11 to 20</u>	<u>95-21 to 30</u>	<u>99-21 to 30</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</u> <u>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	<2.00	<2.00	<2.00	~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

TABLE XI

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>Notebook #16928</u>	<u>93-1 to 10</u>	<u>93-11 to 20</u>	<u>93-21 to 30</u>	<u>98-1 to 10</u>	<u>98-11 to 20</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 (0-65)	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	<2.00	<2.00	~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 XII

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>Notebook #16928</u>	<u>100-1 to 10</u>	<u>100-11</u>	<u>100-12 to 21</u>	<u>100-22 to 31</u>	<u>101-1 to 10</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>% Ash @ 2500°F</u>					
<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	<2.00	<2.00	<2.00	<2.00	~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

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APPENDIX I

Mutagenic Activity in the Salmonella/Microsome Assay (Ames Test) - Materials and Methods

Introduction:

The Ames Salmonella/microsome assay was used to evaluate synthetic crude oils and their distillation fractions for mutagenicity.

Materials and Methods:

The ability of each test sample to revert Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 from histidine dependence to histidine independence is determined.¹

The assay is performed in the presence and the absence of a rat-liver homogenate activation system. In the absence of an activation system, 0.1 ml of a solution of the test sample and approximately 10^8 bacteria in 0.1 ml are added to 2 ml of top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). These components are mixed and poured on the surface of a plate containing 20 ml of Davis minimal agar. To treat in the presence of an activation system 0.5 ml of S-9 mix is added to the bacteria-test sample-top agar mixture. S-9 is the 9,000 x g supernatant of liver homogenate from rats given 500 mg of Aroclor 1254/kg five days before sacrifice. The S-9 mix contains per ml: 0.3 ml of S-9, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 mM sodium phosphate (pH 7.4). The S-9 mix is added to the bacteria, test sample and top agar. These components are mixed and immediately poured over the minimal agar plate. The revertant colonies are counted after the plates are incubated at 37°C for 48 hr.

The cytotoxicity of each test sample in the presence and absence of an activation system, as measured in strain TA 1535, is the basis for selecting concentrations to be used in the mutagenesis experiment. The protocol used to determine the cytotoxicity is identical to the mutagenesis protocol except that 10^3 rather than 10^8 bacteria are used per plate and a nonlimiting concentration of histidine is present. Concentrations of test sample that are nontoxic and, if possible, slightly toxic are selected for the mutagenesis assay.

¹ Ames, McCann and Yamasaki, Mutation Res. 31:347-364, 1975.

If no mutagenic or cytotoxic effect is observed in the standard plate incorporation assay, a spot test is performed in the presence and absence of the activation system using the five tester strains. Bacteria, top agar, and S-9 mix are handled as described above, and are poured over the minimal agar plate. An aliquot of test sample is applied directly to the top agar surface. The number and distribution of revertant colonies is determined after the plates are incubated at 37°C for 48 hr.

Positive (known mutagens dissolved in DMSO) and negative (solvent-DMSO) controls are included in each assay.

Test solutions of each sample are prepared in dimethylsulfoxide (DMSO) to a maximum concentration of 10,000 µg/0.1 ml. Heating and prolonged stirring of test solutions are used to maximize solubility.

Statistical evaluations of the data are used to classify a test sample as mutagenic or nonmutagenic. The total revertant colony number at each treatment condition is compared independently with the total revertant colony number for the appropriate negative control.* Significance is judged at the 0.01 probability level.

*When the sum of control and treatment revertant numbers is less than or equal to eighty, the significance of the difference between the total counts from the two conditions is tested by referring to tables based on the Poisson distribution. When the sum of control and treatment revertant numbers is greater than eighty, the significance of the difference between the total counts from the two conditions is tested using a Z-test based on the normal approximation to the Poisson distribution where

$$Z = \frac{N_2 - N_1}{\sqrt{N_2 + N_1}} \quad (N_2 = \text{total revertant colony number at treatment condition; } N_1 = \text{total revertant colony number on control}).$$

When $Z \geq 2.57$, the treatment value is significantly different from the control value at the 0.01 probability level.

These statistical methods were chosen to judge significance, because historical controls were found to follow a Poisson distribution.

The existence of a dose response is tested by the Spearman rank correlation. Significance is judged at the 0.05 probability level.

A test sample is classified as mutagenic if both a statistically significant increase in total revertant colony number ($p \leq 0.01$) and a dose response ($p \leq 0.05$) are observed.

When a test sample is mutagenic the highest single average number of revertants observed within each trial is expressed as a multiple of the control value for the sensitive strain(s). The number of revertants/nmole or μg of test sample is calculated from the slope of the line determined by linear regression analysis of the data before activity plateaus or decreases.

APPENDIX II

Based on the statistical criteria outlined in Appendix I, the following test samples were found to be nonmutagenic by the Ames Test.

Haskell No.

12760	South Louisiana Petroleum Naphtha
12761	" " " Light Gas Oil
12766	H Coal Syncrude Naphtha
12765	" " " Atmospheric Still Overhead
12767	" " " Light Gas Oil
12770	Paraho Shale Oil Naphtha
12771	" " " Light Gas Oil
12776	Geokinetics in situ Shale Oil Naphtha
12777	" " " " " Light Gas Oil

APPENDIX III

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

<u>Name</u>	<u>Strain</u>	<u>Activation System</u>	<u>Activity</u>
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
Coal Liquids, H-Coal - Atmospheric Still Bottoms	TA 98	+	37.0-40.7 times the spontaneous reversion frequency (2.5-3.0 rev/ μ g)
	TA 100	+	3.4-4.6 times the spontaneous reversion frequency (0.5-0.8 rev/ μ g)
Coal Liquids, H-Coal - Vacuum Still Overheads	TA 1537	+	16.3-36.3 times the spontaneous reversion frequency (0.5-0.6 rev/ μ g)
	TA 1538	+	70.0-105.3 times the spontaneous reversion frequency (3.6-5.4 rev/ μ g)
	TA 98	+	60.1-84.9 times the spontaneous reversion frequency (4.9-7.4 rev/ μ g)
	TA 100	+	4.8-9.0 times the spontaneous reversion frequency (0.9-1.3 rev/ μ g)
Shale Oil, Paraho - Mid Gas Oil	TA 1537	-	2.2-2.3 times the spontaneous reversion frequency (0.01 rev/ μ g)
	TA 100	-	1.3 times the spontaneous reversion frequency (0.04-0.08 rev/ μ g)
	TA 1537	+	4.5-11.6 times the spontaneous reversion frequency (0.03-0.06 rev/ μ g)
	TA 1538	+	5.5-7.4 times the spontaneous reversion frequency (0.2 rev/ μ g)
	TA 98	+	6.6-6.7 times the spontaneous reversion frequency (0.2 rev/ μ g)
	TA 100	+	1.6-2.0 times the spontaneous reversion frequency (0.4 rev/ μ g)*

*Questionable dose response: however, activity is consistent with other test results

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

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

APPENDIX IV

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 2.0 mg test material/0.5 ml toluene at start of experiment. Two weeks later begin painting 2.5 µg phorbol myristate acetate (PMA)/0.1 ml acetone 3 times per week (M, W, F) for six months. Positive control 7,12-dimethylbenzanthracene 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.

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

APPENDIX V

DOE SPONSORED MOUSE SKIN PAINTING INITIATION/PROMOTION STUDY

Date: _____

Day of Promotion _____

<u>Group</u>	<u>Sample</u>
I	Toluene/Acetone 50 µl toluene
II	Toluene/PMA 50 µl toluene
III	None/PMA not treated
IV	benzo(a)pyrene (H-11,269) 50 µg in 50 µl acetone
V	H-Coal FO-ASO (H-13,170) 50 µl as rec'd.
VI	H-Coal FO-ASB (H-13,171) 50 µl as rec'd.
VII	H-Coal FO-VSO (H-13,172) 50 µl as rec'd.
VIII	H-Coal FO-VSB (H-13,173) 50 µl 33% slurry in toluene
IX	H-Coal S-VSB (H-13,174) 50 µl 33% slurry in toluene
X	Occidental Shale Crude (H-13,175) 50 µl as rec'd. 100% warmed
XI	Occidental Shale Naphtha (H-13,176) 50 µl as rec'd. 100% warmed
XII	Occidental Shale LGO (H-13,177) 50 µl as rec'd. 100% warmed
XIII	Occidental Shale MGO (H-13,178) 50 µl as rec'd. 100% warmed in hot H ₂ O
XIV	Occidental Shale Residue (H-13,179) 50 µl 100% warmed in hot H ₂ O
XV	Exxon Donor Solvent Naphtha (H-13,180) 50 µl as rec'd. 100%
XVI	Exxon Donor Solvent Solvent (H-13,181) 50 µl as rec'd. 100%
XVII	Exxon Donor Solvent VGO (H-13,182) 50 µl 33% slurry in toluene
XVIII	Exxon Donor Solvent HGO (H-13,183) 50 µl 100% warmed in hot H ₂ O
XIX	Exxon Donor Solvent Residue (H-13,184) 50 µl 33% slurry in toluene
XX	SRC-II Naphtha (H-13,185) 25 µl 66% soln in toluene
XXI	SRC-II Fuel (H-13,186) 50 µl as rec'd. 100%
XXII	SRC-II VTB (H-13,187) 50 µl 33% slurry in toluene
XXIII	SRC-II STB (H-13,188) 50 µl 33% slurry in toluene
XXIV	SRC-II HGO (H-13,189) 50 µl as rec'd. 100% warmed in hot oil
XXV	SRC-II Residue (H-13,190) 50 µl as rec'd. 33% slurry in toluene