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SYNTHETIC CRUDE OILS CARCINOGENICITY SCREENING TESTS

PROGRESS REPORT

Covering the Period September 15, 1979 to March 15, 1980

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ABSTRACT

Four crude oils (H Coal-Fuel Oil Mode, Occidental in situ Shale Oil, Exxon Donor Solvent Liquid, and SRC II) which were distilled into four fractions (naphtha, mid-distillate, gas oil and residue) for analysis and biological screening testing during the last 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 naphtha fractions of the 4 crude oils showed little or no mutagenicity or 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 fractions.

Syncrude Carcinogenicity Screening Tests
Technical Progress Report for the Period
September 15, 1979 - March 15, 1980

INTRODUCTION

This Report is the third 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 carcinogenic potential that exists in the production, handling and utilization of synthetic crude oils and distillation fractions derived from them. The study involves conducting a series of screening tests for mutagenicity and tumor initiation 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)) are designed to provide a preliminary indication of which materials are potentially more biologically active and should be investigated more thoroughly by longer term testing procedures.

The project is being run in two parts; in each part, four crude oils were processed. The first four samples (S. Louisiana crude petroleum, H coal Syn crude, Paraho Shale Oil and Geokinetics in situ Shale Oil) were distilled and analyzed in a previous report period and the analytical results were reported in the first Progress Report. The biological test results for these four samples were reported in the second Progress Report.

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 results of the fractionation and analysis of these materials were included in the second progress report. The biological test results for these four samples are reported herein.

In all cases except the petroleum controls, samples tested were obtained from small scale experimental units (PDU's) and may or may not be similar chemically and in biological activity to future commercial production.

I. Phase II

A. Biological Testing of the Second Crude Oil Series (Exxon Donor Solvent Liquid, SRC-II, H Coal in the Fuel Oil Mode, and Occidental in situ Shale Oil)

As in Phase I, two types of accelerated biological tests were used to screen these synthetic crude oils and their distillation fractions:

Salmonella/microsome assay

Mouse skin initiation/promotion test

The tests have been completed and reported on the crude oils processed under Phase I. All of the samples in Phase II were distilled into four fractions as described in Progress Report I, except where noted. 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 (dimethyl sulfoxide) as solvent (1). The ability of each test sample to revert, Salmonella typhimurium strains TA 1535, 1537, 1538, 98 and 100 from histidine dependence 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. Summaries of the 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 (naphthas) of all four crude oils tested were found to be nonmutagenic. All the higher boiling fractions 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 higher boiling fractions derived from coal were more mutagenic than the higher boiling fractions from shale oil.

It is interesting to note that the strains of Salmonella with which activity was most frequently observed with the crude oils and fractions under study were TA 98, TA 1537, and TA 1538 in the presence of the activation system.

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 0.1 ml of the sample either as is or in a toluene paste to one square inch shaved patches on the backs of 30 Swiss ICR/Ha female 6 week old mice. Beginning two weeks later, phorbol myristate acetate in acetone was applied three times per week for six months.

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 II crude oils and fractions. Examination of this Table shows very slight or no tumor initiating activity for the low boiling fraction (naphthas) of the four crude oils tested. However, the high boiling fractions and the crude oils themselves were active. Of the active high boiling fractions and crude oils, the coal derived materials were the most active. The shale oil crude and high boiling fractions were similar in tumor initiating activity to the petroleum crude and high boiling fractions run in Phase I.

TABLE I

SUMMARY: Ames Test Results for Phase II Samples

Exxon Donor Solvent Process*

naphtha	nonmutagenic
solvent	mutagenic
vacuum gas oil (VGO)	mutagenic
heavy gas oil (HGO)	mutagenic
residue	mutagenic

H Coal Liquid Fuel Oil Mode*

atmospheric still overhead (ASO)	nonmutagenic
atmospheric still bottoms (ASB)	mutagenic
vacuum still overhead (VSO)	mutagenic
vacuum still bottoms (VSB)	mutagenic

SRC-II*

naphtha	nonmutagenic
fuel oil	mutagenic
heavy gas oil (HGO)	mutagenic
stripper tower bottoms (STB)	mutagenic
vacuum tower bottoms (VTB)	mutagenic
residual oil	mutagenic

Occidental in situ Shale Oil

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

*These sample suppliers submitted production fractions and not whole crudes. Since these generally corresponded to the distillation ranges used in this study, it was decided to test these fractions instead of combining them and redistilling them. This provided information concerning the potencies of the production fractions as well as those of the boiling ranges of interest.

TABLE II

Mouse Skin Painting Initiation/Promotion Study

<u>Sample</u>	<u># Mice With Tumors >30 days</u>	<u>Avg. # Tumors/Mouse</u>	<u>Day 1st Mouse with Tumor</u>
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
VSF	13	2.4	37
Occidental Shale Oil			
Naphtha	1	0	135
LGO	1	2.0	84
MGO	4	1.3	98
Crude	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	63
Residue	21	3.0	51

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.

TABLE III
Comparison of In Vitro and In Vivo Tests

H Coal - Fuel Oil Mode

	<u>AMES TEST (in vitro)</u>					<u>INITIATION/PROMOTION TEST (in vivo)</u>		
	<u>STRAIN (with activation)</u>					<u>mice w/tumors</u>	<u>avg. # tumors</u>	<u># of days</u>
	<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>	<u>lasting >30 days</u>	<u>per mouse</u>	<u>to first mouse</u>
								<u>w/tumor</u>
Atmospheric still overhead	-	-	-	-	-	2	1.0	56
Atmospheric 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	.02 (2.3)	.25 (41.0)	1.6 (66.3)	1.8 (65.7)	1.0 (8.4)	24	4.5	37
Vacuum still bottoms	.03 (2.7)	.55 (61.8)	2.4 (101.8)	2.3 (79.2)	1.2 (17.4)	13	2.4	37

*Revertants per μg (average). Comparisons of revertant(s) per μg 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 III

Comparison of In Vitro and In Vivo Tests

H Coal - Fuel Oil Mode

AMES TEST (in vitro)

STRAIN (without activation)

	<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>
Atmospheric still overhead	-	-	-	-	-
Atmospheric still bottoms	-	-	-	-	-
Vacuum still overhead	-	-	-	-	-
Vacuum still bottoms	-	-	0.003 (2.2)	0.01 (3.2)	0.09 (3.0)

TABLE IV
Comparison of In Vitro and In Vivo Tests

Occidental Shale Oil (in situ)

	AMES TEST (in vitro)					INITIATION/PROMOTION TEST (in vivo)		
	STRAIN (with activation)					mice w/tumors lasting >30 days	avg. # tumors per mouse	# of days to first mouse w/tumor
	TA 1535	TA 1537	TA 1538	TA 98	TA 100			
Naphtha	-	-	-	-	-	1	0	135
Light Gas Oil	-	-	-	.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 (7.7)	0.08 (2.6)	4	1.3	98
Residue	-	0.005 (8.1)	0.025 (7.8)	0.03 (9.1)	0.02 (2.45)	3	1.7	98
Crude	-	0.01 (3.9)	0.036 (6.8)	0.045 (8.2)	0.09 (2.5)	0	0	-

*Revertants per μg (average). Comparisons of revertant(s) per μg 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 TestsOccidental Shale Oil (in situ)AMES TEST (in vitro)STRAIN (without activation)

	<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>
Naphtha	-	-	-	-	-
Light Gas Oil	-	-	-	-	-
Mid-Gas Oil	-	-	-	0.02 (2.8)	0.06 (1.6)
Residue	-	0.001 (2.6)	-	-	0.006 (1.6)
Crude	-	-	-	-	-

TABLE V
Comparison of In Vitro and In Vivo Tests

Exxon Donor Solvent Process (coal)

	<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>avg. # tumors per mouse</u>	<u># of days to first mouse w/tumor</u>
	<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>			
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 (6.2)	18	3.6	42
Vacuum Gas Oil	0.08 (4.1)	0.79 (182.8)	3.13 (134.2)	3.2 (110.7)	2.18 (20.2)	20	3.3	51
Residual Oil	0.05 (4.3)	0.78 (151.3)	4.45 (93.0)	4.6 (71.9)	1.68 (13.3)	17	2.4	42

*Revertants per μg (average). Comparison of revertant(s) per μg 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 Tests

Exxon Donor Solvent Process (coal)

AMES TEST (in vitro)

STRAIN (without activation)

	<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>
Naphtha	-	-	-	-	-
Solvent	-	-	-	-	-
Heavy Gas Oil	-	-	-	-	1.87 (1.87)
Vacuum Gas Oil	-	-	-	0.26 (2.2)	1.81 (2.3)
Residual Oil	-	-	-	1.7 (2.2)	0.86 (1.6)

TABLE VI
Comparison of In Vitro and In Vivo Tests

	<u>SRC II</u>					<u>INITIATION/PROMOTION TEST (in vivo)</u>		
	<u>AMES TEST (in vitro)</u>							
	<u>STRAIN (with activation)</u>							
	<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>	<u>mice w/tumors lasting >30 days</u>	<u>avg. # tumors per mouse</u>	<u># of days to first mouse w/tumor</u>
Naphtha	-	-	-	-	-	3	1.0	63
Fuel Oil	-	0.13 (18.3)	1.11 (22.3)	1.16 (18.8)	0.93 (2.55)	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 (83.3)	2.5 (140.5)	2.9 (77.7)	1.23 (10.4)	15	2.5	51
Vacuum Tower Bottoms	0.02 (3.1)	0.36 (88.0)	1.3 (109.3)	1.34 (60.8)	0.94 (12.3)	10	1.9	63
Residue	0.08 (3.2)	1.4 (117.3)	4.7 (149.3)	4.5 (87.7)	2.9 (13.1)	21	3.0	51

*Revertants per μg (average). Comparisons of revertant(s) per μg 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.

TABLE VI

Comparison of In Vitro and In Vivo Tests

SRC II

AMES TEST (in vitro)

STRAIN (without activation)

	<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 1538</u>	<u>TA 98</u>	<u>TA 100</u>
Naphtha	-	-	-	-	-
Fuel Oil	-	-	-	-	-
Heavy Gas Oil	-	-	-	-	-
Stripper Tower Bottoms	-	0.01 (3.9)	0.04 (4.1)	0.04 (2.3)	0.26 (2.4)
Vacuum Tower Bottoms	-	0.02 (3.5)	0.01 (2.7)	0.03 (2.7)	0.1 (2.4)
Residue	-	0.03 (3.2)	0.03 (2.3)	0.05 (2.7)	0.17 (2.3)

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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.

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 10^5 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.

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

Judged on the basis of the statistical criteria outlined in Appendix I, the following test samples are not mutagenic in the assay:

H-Coal fuel oil mode; atmospheric still overhead

Occidental shale oil; naphtha

Exxon donor solvent; naphtha

SRC II; naphtha

APPENDIX III

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

Name	Strain	Activation System	Activity
H-Coal fuel oil mode; atmospheric still bottoms	TA 1537	+	9.5-16.6 times the spontaneous reversion frequency (0.12-0.16 rev/ μ g)
	TA 1538	+	42.8-56.3 times the spontaneous reversion frequency (0.99-1.48 rev/ μ g)
	TA 98	+	31.0-44.7 times the spontaneous reversion frequency (0.92-1.8 rev/ μ g)
	TA 100	+	3.7 times the spontaneous reversion frequency (0.42-0.48 rev/ μ g)
H-Coal fuel oil mode; vacuum still overhead	TA 1535	+	2.3 times the spontaneous reversion frequency (0.02 rev/ μ g)
	TA 1537	+	22.0-59.9 times the spontaneous reversion frequency (0.12-0.38 rev/ μ g)
	TA 1538	+	50.4-82.2 times the spontaneous reversion frequency (1.1-2.0 rev/ μ g)
	TA 98	+	52.5-78.8 times the spontaneous reversion frequency (1.3-2.2 rev/ μ g)
	TA 100	+	5.2-11.6 times the spontaneous reversion frequency (0.54-1.4 rev/ μ g)
H-Coal fuel oil mode; vacuum still bottoms	TA 1538	-	2.2 times the spontaneous reversion frequency (0.003 rev/ μ g)
	TA 98	-	2.9-3.4 times the spontaneous reversion frequency (0.01 rev/ μ g)
	TA 100	-	2.5-3.5 times the spontaneous reversion frequency (0.06-0.11 rev/ μ g)
	TA 1535	+	2.4-2.9 times the spontaneous reversion frequency (0.01-0.06 rev/ μ g)
	TA 1537	+	37.2-86.3 times the spontaneous reversion frequency (0.35-0.74 rev/ μ g)

Name	Strain	Activation System	Activity
H-Coal fuel oil mode; vacuum still bottoms (continued)	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 (0.14-0.50 rev/ μ g)
	TA 1535	+	2.0-5.5 times the spontaneous reversion frequency (0.05-0.08 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; medium 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)
	TA 100	+	2.2-2.9 times the spontaneous reversion frequency (0.04-0.12 rev/ μ g)
Occidental shale oil; residual oil	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 (0.003-0.007 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 (1.06-3.3 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)
	TA 1537	+	14.3-22.2 times the spontaneous reversion frequency (0.09-0.17 rev/ μ g)
Exxon donor solvent; fuel oil	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 (0.93-1.4 rev/ μ g)
	TA 100	+	2.5-2.6 times the spontaneous reversion frequency (0.80-1.06 rev/ μ g)
	TA 1537	-	2.7-4.3 times the spontaneous reversion frequency (0.01-0.02 rev/ μ g)
SRC 11; vacuum tower bottoms	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 (continued)	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 (0.05-0.05 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)
	TA 100	+	6.4-14.3 times the spontaneous reversion frequency (0.65-1.8 rev/ μ g)

Name	Strain	Activation System	Activity
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)

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 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 myristate acetate (PMA)/0.1 ml acetone 3 times per week (M,W,F) for 180 days. Positive control benz(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