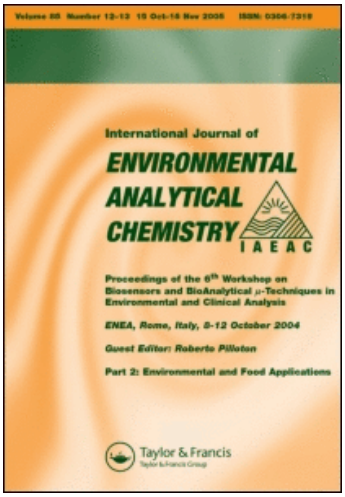

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Separation and Identification of Mutagenic Constituents of Petroleum Substitutes†

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A study combining chemical separations, mutagenicity testing, and spectroscopic identifications is underway to isolate and identify mutagens in coal- and shale-derived oils. Ether-aqueous partition combined with Sephadex LH-20 chromatography of the resulting neutral fraction is introduced as a preferred class fractionation procedure. The uniquely important role of polycyclic aromatic primary amines in the mutagenicity of petroleum substitutes is reviewed. Questions are raised concerning the role of polycyclic aromatic hydrocarbons in the mutagenicity of the neutral fraction of petroleum substitutes.

INTRODUCTION

Petroleum substitutes produced from coal and shale are becoming more important as world petroleum reserves become smaller. Renewed attention is being given to technologies which convert solid fossil fuels to gaseous and liquid products to be used for heating, electric power generation, and as transportation fuels. Similar technologies are being developed to produce solid fuels containing smaller quantities of sulfur and mineral matter than the starting material. To be viable, the processes developed must be economically sound and environmentally acceptable.

Studies are underway here[1–6] to establish the toxicological properties of petroleum substitutes and to determine[5–10] the nature of the constituents responsible for the observed bioactivities. Special attention is being given to the mutagenicity of petroleum substitutes as measured using the Ames test.[11] The approach[12] involves separating the sample by various class

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fractionation methods and subjecting the resulting fractions to mutagenicity testing. Total mutagenicity is estimated by summing those of the individual fractions while the distribution of the mutagenicity provides insight into the nature of the bioactive constituents.

EXPERIMENTAL

Samples and experimental methods are detailed elsewhere.[1,2,5,7–10] Briefly, the petroleum substitutes are first subjected to overnight evaporation at ~35 Torr and room temperature in a vacuum manifold and a volatile matter is collected in dry ice-acetone cold traps. The nonvolatile fraction is partitioned between diethyl ether and 1 N HCl to separate alkaline constituents. The ether phase is then partitioned against 1 N NaOH to remove acidic components. The remaining neutral fraction is subjected to Sephadex LH-20 chromatography using isopropanol to elute aliphatic (saturate), aromatic, and polyaromatic fractions. The column is then washed with benzene to remove residual (neutral-polar) constituents.

Mutagenicity is determined using the standard[1,11] plate assay with *Salmonella typhimurium* strain TA98. Aroclor 1254 induced rat liver S-9 was used to provide metabolic activation for all of the tests reported here. Each fraction is subjected to mutagenicity assay at four concentrations and the activity is derived from the slope of the induction curve. Results are expressed as the number of revertant colonies per milligram of test material.

RESULTS AND DISCUSSION

Samples considered here were provided through the USEPA/USDOE Synfuels Research Materials Facility[13] for generic research into the chemical and biological properties of petroleum substitutes. Their history is not sufficiently documented to provide process-specific conclusions. Petroleum 5107 is a composite containing six different crudes and 5301 is a single crude oil designated as "Wilmington, CA". Shale oil 4101 is known to be from a simulated in-situ experimental scale liquefaction of shale and 4601 from an above-ground retorting process. Coal oil 1202 is a crude product oil, 1106 is a partially hydrotreated oil, and 1701 is termed a fuel oil blend. Coal oils 1309 and 1310 are described as atmospheric still bottoms and vacuum still overheads, respectively. Different processing technologies were used to generate each of the oils except for 1309 and 1310 which come from the same process.

Figure 1 illustrates the general class fractionation method used at this laboratory to track mutagens. Volatile matter is first removed by distil-

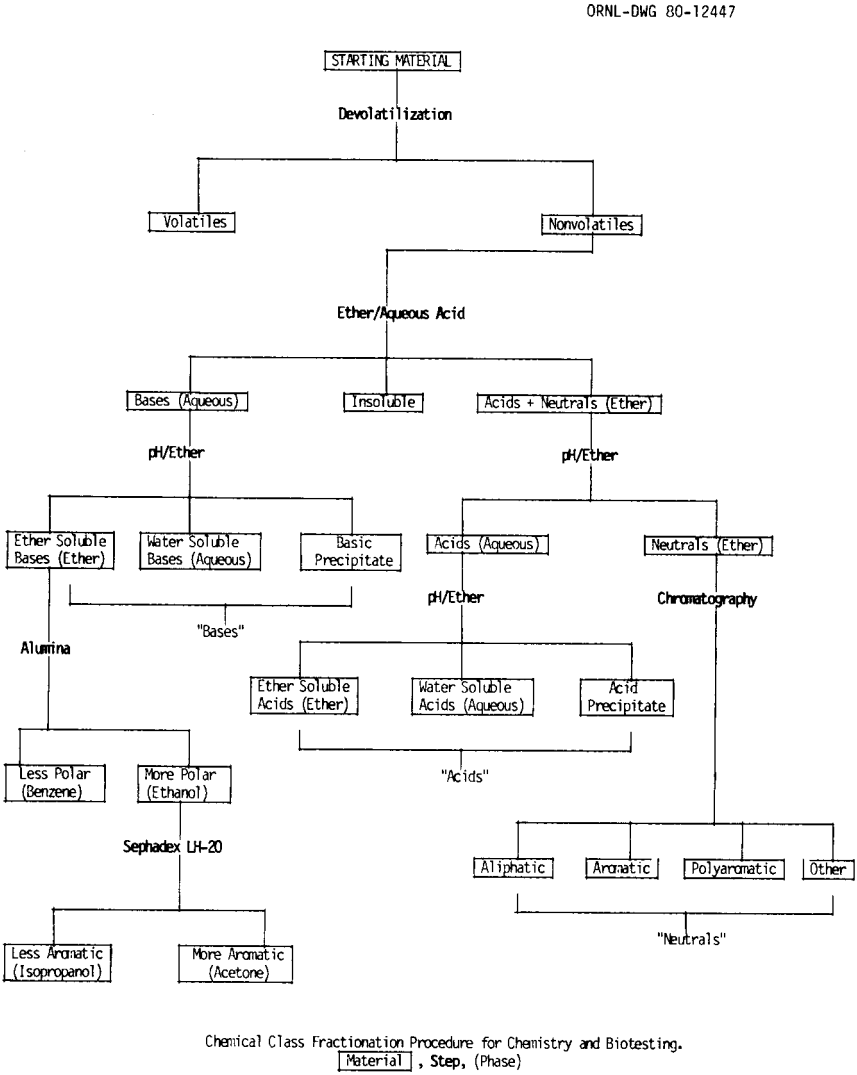

FIGURE 1 Chemical class fractionation procedure for chemistry and biotesting. MATERIAL STEP, (PHASE).

lation[14] or evaporation[5] and the residue is separated[15] into acidic, basic, and neutral fractions. Material insoluble in the initial ether/acid mix, precipitates formed upon pH adjustment, and material remaining in the aqueous phase upon back extraction of acidic and alkaline constituents are collected for testing and material balance calculation. The neutral

fraction, representing the majority of the starting material (Table 1), is generally subfractionated[1,2,15] by adsorption column chromatography. The subfractionation (Figure 1) of the ether soluble base fraction is not required for biological testing but is found[7] to yield a ten-fold enrichment of the mutagens which is helpful for detailed chemical analysis.

A similar method of class fractionation has been reported by others.[16] The sample is partitioned between iso-octane and aqueous phases to yield the acidic, alkaline, and neutral fractions. The neutral fraction is subsequently enriched in aromatics by partitioning between iso-octane and DMSO. Insoluble matter is collected as "tars" and is subjected to biotesting.

Sephadex LH-20 has been shown[14] to separate the lipophilic fraction of petroleum substitutes by aromaticity when isopropanol is used as the eluent. We find the procedure equally well suited to separating the neutral fraction. The neutral fraction is separated into aliphatic (saturates), aromatic, polyaromatic, and polyaromatic polar constituents with good reproducibility. The column may be used repeatedly without deterioration. Further, losses of constituents due to irreversible adsorption on the column or reaction with the column packing are thought to be much less likely than with adsorption columns.

Distribution of Mutagenicity by Chemical Class. We have previously reported[1,2,15] the distribution of mutagenicity as determined by ether/aqueous partition and adsorption chromatography. Substituting LH-20 chromatography yields (Table 1) comparable results as regards the distribution of mutagens. Alkaline and neutral constituents are seen to contribute to the mutagenicity. Alkaline constituents are uniquely important for the petroleum substitutes because of their inordinately high contribution to mutagenicity relative to their mass contribution.

More detailed (Table II) analyses of the data reveals that the ether soluble basic (ESB) subfraction and the neutral-polyaromatic (N-Ply) and neutral-polar (N-Pol) subfractions contain the principal contributors to mutagenicity. The ESB fraction frequently exhibits the highest specific activity. The N-Ply fraction contributes a greater amount to the total mutagenicity primarily because it constitutes a greater percentage of the mass of the sample.

Alkaline Mutagens. The ESB fraction has been[7] further purified by basic alumina column chromatography using benzene and ethanol as eluants. The ethanol eluate is subsequently eluted through Sephadex LH-20 using isopropanol followed by acetone. Approximately 90% of the mutagenicity of the ESB fraction was accounted for[7] in the acetone subfraction which compromised only 10% or less of its mass.

TABLE I

Mutagenicity and weight distribution by major chemical class fraction

Sample	Mutagenicity distribution					Weight distribution				
	Total rev/mg	Acids %	Bases %	Neutrals %	Other %	Acids %	Bases %	Neutrals %	Other %	Recovery %
Petroleum[a] 5107[b]	150	2	3	95	0	1	2	85	1	89
Petroleum 5301	5	0	0	100	0	2	1	90	7	99
Shale Oil[a] 4101	180	2	42	54	2	2	7	85	1	
Shale Oil 4601	390	0	69	31	0	1	4	95	0	100
Coal Oil[c] 1202	4200	3	80	10	8	8	10	65	20	103
Coal Oil[c] 1106	530	0	11	89	0	4	7	85	1	97
Coal Oil 1701	1100	0	35	65	0	4	5	80	3	92
Coal Oil 1309	970	1	46	53	0	<1	3	95	<1	99
Coal Oil 1310	2400	0	42	58	–	1	3	95	1	100

[a]Reference 1.
[b]USEPA/USDOE Synfuels Research Material Facility (13) designation.
[c]Reference 2.

TABLE II

Mutagenicity and weight distribution by subfractions[a]

Subfraction	Specific mutagenicity[b]					Weighted mutagenicity[c]					Weight distribution[d]				
	Petr 5301	Shale 4601	Coal 1309	Coal 1310	Coal 1710	Petr 5301	Shale 4601	Coal 1309	Coal 1310	Coal 1710	Petr 5301	Shale 4601	Coal 1309	Coal 1310	Coal 1710
Volatiles	0	0	0	0	0	0	0	0	0	0	7	<1	<1	<1	3
Ether soluble acids	0	0	1200	700	—	0	0	4	4	0	<1	<1	<1	<1	4
Acid insolubles	0	0	0	200	0	0	0	0	1	0	2	<1	—	—	<1
Ether soluble bases	0	8000	22500	40000	6000	0	265	445	988	349	<1	3	2	2	5
Base insolubles	0	141	643	600	0	0	1	5	3	0	<1	<1	<1	<1	<1
Neutral-saturates	0	20	12	40	104	0	11	2	4	5	56	56	20	10	5
Neutral-aromatics	20	0	0	0	0	4	0	0	0	0	17	27	63	64	60
Neutral-polyaromatics	15	1600	5000	6000	10000	2	111	513	1367	748	10	7	21	23	7
Neutral-polars	0	0	3000	1800	800	0	0	1	1	1	6	2	<1	<1	<1
Total[e]	—	—	—	—	—	6	388	970	2368	1070	99	97	97	100	84

[a]See also Reference 5.

[b]Number of revertant colonies per milligram of fraction.

[c]Number of revertant colonies per milligram of starting sample contributed by the fraction.

[d]Weight percentage.

[e]By summation of subfractions.

The acetone subfraction was analyzed[7] by gas chromatography, infrared spectroscopy, and mass spectroscopy. Gas chromatographic profiles indicated the presence of up to one hundred constituents and mass spectrometric data indicated the presence of polycyclic aromatic nitrogen-containing constituents. Infrared spectra suggested the presence of primary amino groups. The acetone eluate was subjected to trifluoroacetylation and the resulting sample was again gas chromatographed. Shifts in retention times showed that most of the constituents had been derivatized further suggesting the presence of polycyclic aromatic primary amines. The likely importance of primary amines was confirmed by testing several authentic pure compounds which gave the following results: 2-aminoanthracene, 200,000 rev/mg; 3-aminopyrene, 2,600,000 rev/mg; 3-aminoperylene, 200,000 rev/mg.

The acetone subfraction has been subjected to higher resolution Sephadex LH-20 chromatography using isopropanol to provide a crude fractionation by ring number. The mutagenicity was found to be greatest in the 3–4 ring range with significant activity also appearing in the 2–3 and >4 ring ranges. The possible contribution of secondary and tertiary amines to the mutagenicity of the acetone eluate was ruled out with the finding that such compounds accumulate in the benzene eluate of the alumina column separation.

The importance of polycyclic aromatic primary amines is also suggested[17] by a recent report of their contribution to the mutagenicity of a fuel oil blend from the Solvent Refined Coal Process. The observation is based upon thin layer chromatographic isolation and mass spectrometric identification.

Neutral Mutagens. Previous studies of the neutral fraction have addressed the roles of aromaticity,[10] polycyclic aromatic alkyl derivatives,[9] and neutral azaarenes.[8] It is generally found that mutagenicity concentrates in the fractions containing polycyclic aromatic constituents. No activity has been observed in the aliphatic fraction or the volatile fraction (containing primarily low molecular weight hydrocarbons). Data has been reported suggesting that mutagenicity is concentrated in the four–five ring subfractions[18] of polycyclic aromatic fraction and that both multi-alkylated and parent polycyclic aromatic hydrocarbons contribute to mutagenicity.

Studies[8] of neutral azaarenes have raised questions about the role of polycyclic aromatic hydrocarbons in the mutagenicity of petroleum substitutes. The neutral fractions of a coal- and shale-derived oil were subjected to Sephadex LH-20 chromatography to separate aliphatics from aromatics. The aromatic fraction was subjected to silicic acid chromatog-

raphy by sequential elution with 1/3 benzene/hexane, 1/2 benzene/hexane, and ethanol as has been described[18] for tobacco smoke condensate analysis. The procedure provided fractions enriched in polycyclic aromatic hydrocarbons, neutral azaarenes, and residual polar materials respectively. The azaarenes were found to contribute slightly to the mutagenicity of the coal oil but insignificantly to that of the shale oil. The polar fraction was found to contribute more (80% for the shale oil, 43% for the coal oil) to the observed mutagenicity than did the polycyclic aromatic hydrocarbon fraction (20% and 35%, respectively).

The study was repeated using additional sample types. Preliminary results (Table III) confirm that greater mutagenicities are often exhibited by the polycyclic aromatic polar constituents than by the hydrocarbons. Mutagenic activity previously ascribed to the polycyclic aromatic hydrocarbons may thus be due to polycyclic aromatics containing polar substituents.

TABLE III

Mutagenicity of neutral subfractions from silicic acid chromatography

Subfractions	Mutagenicity[a]			
	Petr 5301	Shale 4601	Coal 1309	Coal 1310
Neutral	±	++	+	++
Aliphatic	−	−	−	−
Polycyclic aromatic hydrocarbon	±	+	±	±
Polycyclic aromatic nitrogen	±	±	±	±
Polycyclic aromatic polar	±	+	++	++

[a]1000–2000 rev/mg (++), 200–1000 rev/mg (+), 10–200 rev/mg (±), <10 rev/mg (−).

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