Biologically Important Compounds in Synfuels Processes*

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DISCLAIMER

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ABSTRACT

Crude products, by-products and wastes from synfuel processes contain a broad spectrum of chemical compounds--many of which are active in biological systems. Discerning which compound classes are most important is necessary in order to establish effective control over release or exposure. Polycyclic aromatic hydrocarbons (PAH), multi-alkylated PAH, primary aromatic amines and N-heterocyclic PAH are significant contributors to the overall mutagenic activities of a large number of materials examined. Ames test data show that the basic, primary aromatic amine fraction is the most active. PAHs, multi-alkylated PAHs and N-heterocyclic PAHs are all components of the neutral fraction. In nearly all cases, the neutral fractions contribute the largest portion of the mutagenic activity, while the basic primary aromatic amine fractions have the highest specific activity. Neutral fractions are usually the largest (wt%) whereas the total basic fractions are small by comparison; thus, the overall greater contribution of the neutral fraction to the mutagenic activity of most samples. Biologically active constituents are isolated in preparative scale amounts from complex mixtures utilizing combinations of liquid-liquid extraction and various liquid chromatographic column-eluent combinations. Fractions are characterized using a combination of spectroscopic techniques and gas chromatography/mass spectrometry.

INTRODUCTION

The problem of determining the biological activity of a compound is not new. With the growth of governmental regulations to control what is eaten, breathed, touched or is otherwise made available through environmental distribution, answering questions of biological activity has reached new importance. Unfortunately, the ability to ascertain the biological activity of a single compound, especially its long-term effects in humans, is far from straightforward. These remarks are supported by recent experiences with compounds which have long been a part of the industrial environment, e.g., monovinyl chloride and benzene. Clearly, an even greater problem exists in determining the biologically active constituents of a mixture of compounds, especially when the mixture is complex and contains unknown quantities of unknown compounds. Mixtures of this ilk are the products and by-products of synthetic liquid fuels processes.

Chemical and biological characterization of petroleum substitutes and the by-products of their manufacture becomes an increasingly important objective with the projections of a synfuels industry soon to produce one million barrels per day of petroleum substitutes. Development of the industry is impeded by concerns for environmental and occupational safety (1,2). Among the concerns is the threat of an increased incidence of human cancer among those experiencing chronic exposure to synfuel products. These concerns are supported because of the similarity of some synfuel products to known carcinogenic mixtures such as coal tar and because of experiences in a coal conversion plant (3), a gas works facility (4), and in the early chimney sweeping and mule-spinning industries (5).

Concerns over the production of potentially hazardous substances and the need for petroleum substitutes can both be accommodated by applying state-of-the-art techniques to the evaluation of prototype synfuel products. Identification of likely hazardous compounds or classes through systematic chemical/biological studies can provide the information necessary to implement adequate process controls to minimize production of and exposure to pathogens.

This paper summarizes on-going studies which coalesce to address the question of which compounds generated in synfuels processes may be most biologically active. The approach in all of these studies has been to employ separation techniques suitable for generating reasonably reproducible chemical class fractions in large enough quantities for bioassay testing. The most biologically active fractions have become the focus for chemical characterization. Several different bioassays have been used in evaluating synfuel products, but the preponderance of data has been generated from the standard Ames test using the strain TA98 with S-9 activation. These are the only bioassay data reported here.

Our studies of a wide variety of petroleum substitutes from experimental scale processes have produced a data base which shows some consistent trends. Most notably, we find that alkaline constituents of the petroleum substitutes are often major contributors to their mutagenicity. In contrast, the mutagenicities of petroleum crudes is determined almost solely by the neutral constituents as would be expected if polycyclic aromatic hydrocarbons (PAHs) were the determinant constituents. The alkaline mutagens in petroleum substitutes may thus pose a problem unique to the emerging fossil fuels conversion

industry. Neutral fractions of petroleum substitutes are, in most cases, the largest contributor to the overall mutagenicity of the whole material. Although neutral fractions usually have a lower specific activity than alkaline fractions, the much greater amounts of neutral substances increases their total contribution relative to that of the alkaline fraction.

Components of the neutral fractions which we have partially characterized include PAHs, multialkylated PAHs and neutral nitrogen pyrrole homologues. The multialkylated PAH fraction is discussed briefly in this paper; neutral nitrogen compounds have not been sufficiently characterized to determine their biological importance.

Alkaline fractions, being uniquely important in most crude synfuel products, are discussed in some detail. Isolation and analysis of the alkaline mutagens suggests that azaarenes and aromatic primary amines are responsible for the mutagenicity. Polycyclic aromatic primary amines (PAAs) are found to predominate in the mutagenic isolate and are found to exhibit exceptionally high mutagenicities. These results suggest that PAAs should be monitored in occupational and environmental assessment components of technology development programs. They also suggest that engineering steps capable of degrading primary amine functionalities or removing highly alkaline constituents may be effective in reducing the mutagenicity of petroleum substitutes.

MATERIALS AND METHODS

Sample Description

Samples examined in this study were supplied to the USEPA/USDOE Synfuels Research Materials Facility (6) for generic research into the

chemical and biological properties of petroleum substitutes. Process operating conditions at the time of sampling, sampling conditions, and sample histories are not sufficiently defined to allow process-specific conclusions to be drawn from the study of these samples. Synfuels Research Materials Repository numbers are identified for each sample to allow future intercomparisons with other investigators and with previously published results (7,8,9).

The composite petroleum crude oil (Repository number 5107) is made up of 20% Prudhoe Bay, Alaska; 20% Wilmington, California; 20% South Swan Hills, Alberta; 20% Gach Saran, Iran; 10% Louisiana-Mississippi Sweet; and 10% Arabian Light (all in % v/v) crude oils. All but the Louisiana- Mississippi Sweet (LMS) oil (Repository number 5101) were contributed by the Laramie and Bartlesville Energy Technology Centers and have been studied as part of the American Petroleum Institute (API)-60 chemical characterization project. The LMS oil was contributed by J. A. Carter, Analytical Chemistry Division, Oak Ridge National Laboratory. The Wilmington (Repository number 5301) and the Recluse (5305) crude oils were contributed by the Bartlesville Energy Technology Center. Shale-derived crude oil 4101 was produced by the Laramie Energy Technology Center during a simulated insitu retorting in their 150 ton above-ground retort. The reference above- ground retorted shale oil (number 4601) was contributed by the Department of the Navy through its contractors, Development Engineering, Inc., (the Paraho Process) and the Standard Oil Company of Ohio. Coal oil number 1202 was supplied by the Pittsburgh Energy Technology Center from an experimental run of the Synthoil Process. Coal-derived petroleum substitute number 1701 was supplied by Pittsburgh and Midway Mining as a "fuel oil

blend" resulting from the Solvent Refined Coal II Process. Coal-oils number 1309 and 1310 are designated as atmospheric bottoms and vacuum overheads, respectively, produced in the Process Development Units by Hydrocarbon Research, Inc., using the H-Coal Process in the Syncrude mode. Sample number 1106 was contributed by FMC Corporation studying the Char-Oil-Energy-Development (COED) Process. The oil was described as a COED "product" which was hydrotreated to some extent in the process. Information on process characteristics may be found in source documents (10,11).

Chemical Class Fractionation

The simulated in-situ shale oil was supplied as an emulsion of oil and product water. The sample was batch-centrifuged here at 2500 rpm for about 20 minutes at room temperature to break the emulsion. The oily layer (representing approximately 50% by volume of the raw sample) was used for chemical class fractionation. All other samples were fractionated as received.

Each sample was subjected to rotary evaporation at 40°C under water aspirator vacuum until constant weight had been achieved. The remaining oil was (12) slurried in diethyl ether and contacted with 1 N HCl or NaOH in a separatory funnel. Five grams of oil are typically processed using 250 ml of ether and 250 ml of aqueous phase. The aqueous extracts were adjusted to pH 11 or 1, respectively, and were extracted with 250 ml of diethyl ether. The basic or acidic constituents, respectively, were thus back-extracted into ether to yield ether-soluble bases (or acids) and water-soluble bases (or acids) fractions. Precipitates which formed were collected by centrifugation or filtration for further study. The neutral constituents remaining in

the initial ether phase were then separated using basic alumina or Sephadex LH-20 into aliphatic, aromatic, polyaromatic (3+ fused rings), and residuals as has been described (12,13).

Subfractionation of the Multialkylated PAH Fraction

The gross PAH isolate was separated into its multialkylated PAH and simple alkylated plus parent PAH fractions using a gel filtration method (14). The PAH isolate was eluted in two fractions from four 0.5 in. o.d. x 43 in. Biobeads SX-12 columns with redistilled benzene. The same 2,3,5-trimethylnaphthalene/fluoranthene cut-point (14) was used to separate the multialkylated PAH from the parent plus simply alkylated PAH, but collection of the multialkylated PAH fraction commenced with the rise in 280 nm absorbance of the column eluate. The collection of the parent plus simple alkylated PAH fraction was extended through the elution of coronene. The solvent was removed from the two collected fractions by evaporation with dry flowing nitrogen under reduced temperature and pressure. The multialkylated PAH contributed three times more mass to the gross PAH isolate of this coal-derived crude oil than did the parent and simple alkylated PAH.

Subfractionation of Ether Soluble Bases

Ether was removed by room temperature evaporation and the remaining ether soluble bases (ESB) were transferred to a basic alumina column using benzene. One gram of the ESB was typically processed using a 50 ml buret (modified to include a solvent reservoir at the top) to which 40 grams of basic alumina had been added in 75 ml of benzene. The sample was eluted first with 500 ml (in some cases 1500 ml, see "Discussion") of benzene and then 700 ml of ethanol. The ethanol was removed by rotary evaporation and the residue was taken up

in isopropanol. The sample was transferred to a column containing Sephadex LH-20 (75 gm of Sephadex LH-20 in a 250 ml buret compacted by pre-eluting the column with 50-100 ml of isopropanol) and was eluted first with 250 ml of isopropanol and next with 600 ml of acetone. Solvents were removed by rotary evaporation at a water bath temperature of 40°C and 30 torr pressure between steps and prior to preparation of biotesting.

Bacterial Mutagenicity Bioassay

Salmonella typhimurium strain TA98, obtained through the courtesy of Dr. Bruce Ames, Berkeley, California, was used for these assays. The standard procedure (15) involved addition of TA98 to soft agar containing a low level of histidine and biotin along with the fraction being tested. The suspension containing approximately 2×10^8 bacteria is overlaid on minimal agar plates. Rat liver S-9 following Aroclor 1254 induction is incorporated in each plate to provide for metabolic activation. Activity in revertants per milligram (rev/mg) of test substance is derived from the slope of the induction curve extrapolated to milligram values from tests at four concentrations of the test substance. Total rev/mg is computed by summing rev/mg found for each constituent fraction following correction for the weight percentage of the crude material accounted for by the fraction. Individual subfractions (Figure 1) are summed to compute percentage distribution by general chemical class to the total mutagenicity of the crude oils.

Chemical Apparatus and Instrumentation

All solvents were reagent grade and were freshly distilled except for the absolute ethanol. Chromatography was carried out using 50 ml

and 250 ml burets to which glass eluting solvent reservoirs had been attached. The packing was introduced into the buret in the solvent selected for initial elution and approximately one column volume of the solvent was passed through the column to aid settling. Gravity feed and manual fraction collection was employed throughout these studies. For ESB subfractionation, optimum separation conditions were determined by comparing the mutagenicity exhibited by empirically collected fractions. The separation behavior was elucidated and periodically monitored using carbon-14 labelled known compounds of the appropriate classes. No instrumental detector was employed for the preparative column chromatography. Extractions were generally carried out manually using separatory funnels equipped with teflon stopcocks. Solvents were reduced or removed using Brinkmann rotary evaporators with the sample maintained at approximately 40°C by a water bath and either house vacuum or water aspirator pressure (~30 torr).

Gas chromatography of basic fractions was carried out using a 20 ft. x 0.125-in. glass column packed with 3% (wt/wt) Dexsil 400 on 100/120 mesh Supelcoport. The column temperature was programmed from 100°C to 320°C at 1°C/min with a helium carrier gas flow rate of approximately 18/min to elute the constituents of the sample. A Perkin-Elmer Model 3920 gas chromatograph and a DuPont Model 21-490B mass spectrometer with a Hewlett-Packard 21-094B data system interfaced to the 3920 were used for gas chromatographic and mass spectrometric analyses. Studies of PAH and multialkylated PAH fractions utilized glass capillary column gas chromatography. Columns were manufactured in-house and were 30 m x 0.2 mm i.d., coated with Dexsil 400 stationary phase. A Beckman IR-12 was used for infrared spectrometry.

RESULTS AND DISCUSSION

Several petroleum crude oils and petroleum substitutes have been chemically class fractionated (Figure 1) and each fraction has been subjected to mutagenicity bioassay as detailed above. Volatile constituents were first removed and the remaining material was divided into alkaline, acidic, and neutral constituents by acid-base-diethylether solvent partitioning (12). The neutral fraction, generally constituting 80% or more of the weight of the oil, was further separated (13,12) chromatographically into aliphatic-, simple aromatic-, polycyclic aromatic-, and polar-enriched subfractions. The polycyclic aromatic fraction of two coal crudes was separated into multialkylated and simple PAH fractions. The mutagenicities of each subfraction, expressed as the number of revertant colonies per milligram (rev/mg) of test substance, were normalized by weight and summed to compute the mutagenicity of the major (acid, base, neutral) class fractions. The mutagenicities of the major fractions plus those observed for the volatiles and initial precipitate (designated "insoluble" in Figure 1) were summed to compute the mutagenicities of the starting material again, correcting for their weight contributions to the oil. Summation is necessary (8) because most oils and some fractions are incompatible with the bioassay system and cannot be tested directly. Where it has been possible to test both the starting material and its fractions, good additivity is observed (9).

The mutagenicities of the oils are found (Table 1) to vary over several orders of magnitude when comparing petroleum and petroleum

substitutes. Large differences can also be observed within a sample type. Petroleum samples studied here, for example, exhibited (Table 1) mutagenicities of from less than 10 rev/mg to greater than 100 rev/mg. Coal-derived petroleum substitutes exhibited mutagenicities differing by a factor of ten. That this variability is not due to experimental variability is demonstrated by the general agreement between results from repeated class fractionation and bioassay for the same samples (petrolcum number 5107 and petroleum substitutes numbers 1202 and 1106 in Table 1).

Other than noting the general tendency for coal-derived substitutes to exhibit a greater mutagenicity than do petroleum crude oils, it is impossible to uncategorically compare the absolute mutagenicities of petroleum with shale- and coal-derived substitutes based on these samples alone. Additional samples of many differing origins and histories must be tested to provide quantitative generalizations. An important qualitative observation, however, is the large percentage contribution to the mutagenicities of the petroleum substitutes by their alkaline ("basic") fractions. Of particular importance is the finding that petroleum does not exhibit this behavior. The biological properties of petroleum substitutes may thus involve constituents unique to the industry. Another important observation is the highly aromatic character of the neutral fractions of coal-derived crudes relative to petroleum crudes.

Neutral fractions constitute a major portion of all the petroleum substitutes examined; many classes of compounds can be separated from the neutral portion. In particular, the PAH fraction is considered biologically important because of the well documented carcinogenic

properties of several compounds in this class. The coal-derived crudes contain a very complex array of simple (unsubstituted or substituted sparsely with alkyl derivatives) and multialkylated PAHs. A preliminary study (17) of the multilakylated PAH fractions of two (numbers 1202 and 1106) coal-derived crudes was carried out to assess the relative biological importance of parent and simple PAHs to the multialkylated species. Fractions were produced as detailed in the previous section and were analyzed gas chromatographically on a glass capillary column. The profiles obtained for one of the crudes are shown in Figure 2. The gross PAH isolate, the multialkylated PAH fraction and its parent plus simple alkylated PAH fraction were subjected to the Ames test. The mutagenic potential of the multialkylated PAH fractions appeared to be slightly less than that of the corresponding parent plus simple alkylated PAH fractions. However, the total contribution to mutagenicity from the multialkylated fraction is larger than that of the parent plus simple fraction because of the relatively larger amounts of multialkyalted compounds (3-4 times greater by weight).

The ether soluble base (ESB) subfractions of many petroleum substitutes exhibited the highest specific activities (rev/mg of the fraction) and have been the focus of rather detailed study relative to the other fractions. The ESB was further separated by first eluting the fraction through basic alumina with benzene and removing the remaining constituents with ethanol. The ethanol eluate was then separated into isopropanol- and acetone-eluatable fractions using Sephadex LH-20. As described below, it was later found that using an additional volume of benzene (Bz_2 in Table 2) to elute constituents from the alumina column provided a chemically more homogeneous acetone eluate.

Quantitative mutagenicity testing of the ESB subfractions showed (Table 2) that most of the mutagens were concentrated in the acetone eluate. Ninety percent of the mutagenic activity could often be accounted for in the acetone eluate which constituted 10% or less of the weight of the ESB fraction. Further, the acetone eluates of the petroleum substitutes exhibit (Table 2) mutagenic activities similar to or greatly exceeding that (50,000 rev/mg) of pure benzo(a)pyrene. Notably, the acetone subfraction of the petroleum ESBs (which themselves yielded no measurable mutagenic activity) exhibited a slight mutagenicity.

Both the inactive (benzene from alumina and isopropanol from Sephadex LH-20) and active (acetone from LH-20) ESB subfractions of coal oil number 1202 and shale oil number 4101 were subjected (9) to gas chromatographic/mass spectrometric analysis. Each fraction contained 100 or more chromatographable constituents. Mass spectra showed the constituents contained nitrogen (odd mass/charge ratio for parent ions), were highly aromatic, and frequently contained alkyl substituents. Subsequent infra-red spectroscopic analysis of the acetone eluates yielded absorption bands at 3220 cm^{-1} and 3370 cm^{-1} indicating the presence of free amino substituents. Spectroscopic data thus indicate the presence of ring-alkylated polycyclic aromatic primary amines, secondary or tertiary amines, and/or alkylated nitrogen heterocyclics (azaarenes).

The acetone subfractions from both the shale- and coal-derived petroleum substitutes were resubjected to basic alumina column separation using benzene and ethanol to further concentrate the more polar constituents in the ethanol eluate. Mutagenicity testing showed that 93% and 116% of the mutagenic activity was accounted for in the ethanol

isolate for the shale- and coal-derived oils, respectively, while the ethanol eluate constituted 56% and 90% of the weights of the initial acetone subfractions of the oils, respectively. The more polar constituents, e.g. those which would contain free amino-groups rather than ring-nitrogens were thus implicated as the causative mutagens.

To test the hypothesis that aromatic amines were the predominant constituents of the bioactive acetone eluate, the eluate was gas chromatographed before and after reaction with trifluoroacetylimidazole. The reaction converts primary amino groups to trifluoroacetamides but leaves azaarene nitrogen substituents intact. Chromatographic peaks exhibiting the same retention times before and after contact with trifluoroacetylimidazole would be indicative of the presence of non-reactive organonitrogen compounds, i.e. azaarenes. The chromatograms of the initial and and reacted acetone subfractions bore little resemblance to one another suggesting that the great majority of the constituents had reacted to form amides. Further, the derivatized sample was partitioned between acid and ether to back-extract underivatized azaarenes. Almost all of the resulting gas chromatographic peaks from the analysis of the ether-extractable material could be accounted for in the pre-extraction profile, further suggesting that most constituents had been derivatized.

Several azaarenes (C_3-pyridine, quinoline, acridine, a dibenzacridine, and an azabenzo(a)pyrene), tertiary aromatic amines (N, N-dimethylaniline and triphenylamine), secondary aromatic amines (N-methyl-aniline, N-phenylaniline, and N-phenyl-1-naphthylamine), and primary aromatic amines (aniline, 1-naphthylamine, an aminoanthracene, and an aminopyrene) were subjected to basic alumina chromatography

under the conditions used to isolate the mutagens. It was found that the primary amines, beginning with aniline, were retained by the column until 1.5 litres of benzene had been eluted. The azaarenes and the secondary and tertiary amines elute completely before this point is reached. The ESB's of petroleum sample 5301, shale-derived substitute 4101 and coal-derived substitute 1202 were separated using 1.5 l rather than 500 ml of benzene to elute the alumina column in order to provide a polycyclic aromatic primary amine (PAA) fraction and the resulting PAA's were tested (Table 2) for mutagenic activity. Most of the bioactivity was found in the PAA subfraction.

The PAA trifluoroacetylated fraction of coal-derived petroleum substitute number 1202 was subjected to gas chromatographic and mass spectrometric analysis. Approximately 100 constituents were observed (Figure 3) with mass spectral data indicating the presence (Table 3) of a variety of aromatic and polycyclic aromatic primary amines.

The PAA fraction from coal-derived oil number 1202 was finally separated further by ring number using Sephadex LH-20 for a qualitative assessment of the distribution of mutagenic activity. The PAA fraction and its subfractions all exhibited mutagenicity with the activity being greatest for the four-ring constituents but also very high for the 2-, 3-, and 5-ring constituents. As a final test of the hypothesis that primary aromatic amines are the determinant mutagens, several representative pure compounds were tested. Aniline exhibited no activity but the higher amines yielded results as follows: 2-naphthylamine, 70,000 rev/mg; 2-aminoanthracene, 200,000 rev/mg; 3-aminopyrene, 2,600,000 rev/mg; 3-aminoperylene, 200,000 rev/mg. The extraordinarily high

mutagenicities of individual constituents of the acetone subfraction required to account for its high mutagenicity is thus confirmed.

Polycyclic aromatic primary amines may pose environmental health hazards unique to the developing synthetic fuels industry. An awareness of their presence in petroleum substitutes and their unusual mutagenic potential may be used to eliminate their contribution to future environmental and occupational health impacts and to hasten the development of a sorely needed energy technology.

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Table 1. Contribution to Mutagenicity of Oils by Chemical Class

	Mutagenic Activity of Oil				
	Total (Rev/mg)	Percentage of Total (Percent)			
		Neutrals	Acids	Bases	Other
Petroleum Crude Oil					
Composite No.[a] 5107	147	95	2	3	1
Composite No. 5107	241	97	2	2	1
Wilmington No. 5301	5	100	0	0	0
Recluse No. 5305	6	100	0	0	-
LMS No. 5101	75	100	0	0	0
Shale-Derived Petroleum Substitute					
LETC No. 4101	178	54	2	42	2
Paraho No. 4601	388	31	0	69	0
Coal-Derived Petroleum Substitute					
Synthoil No. 1202	4032	9	2	84	7
Synthoil No. 1202	4189	10	3	80	8
SRC II No. 1701	1000	65	0	35	0
H-Coal ASB No. 1309	1230	63	0	37	0
H-Coal YSOH No. 1310	4100	76	0	24	0
COED HDT No. 1106	516	89	0	11	0
COED HDT No. 1106	484	86	0	14	0

[a]USEPA/USDOE Sample Repository Designation.

Table 2. Enrichment of Mutagenic Component of The Ether Soluble Base Fractions

Sample	Specific Activity in Rev/mg					Acetone Subfraction	
	ESB Fxn	Bz_1	Bz_2	Isopr	Acetone	Rev/mg (%ESB)	Wt% ESB
Petroleum							
Composite No.[a] 5107	0	0	-	0	750	>100	5
Wilmington No. 5301	0	0	0	0	850	>100	1
Shale-Derived							
LETC No. 4101	2500	0	-	277	25,000	90	9
LETC No. 4101	1750	120	500	1000	40,000	69	3
Coal-Derived							
Synthoil No. 1202	30,000	850	-	0	220,000	88	9
Synthoil No. 1202	40,000	1750	3000	6000	360,000	90	10
SRC II No. 1701	14,000	0	-	400	67,500	72	15

[a]USEPA/USDOE Sample Repository Designation.

Table 3. Mass Spectral Identification of Gas Chromatographic Eluates from Acetone Subfraction of Synthoil No. 1202 ESB

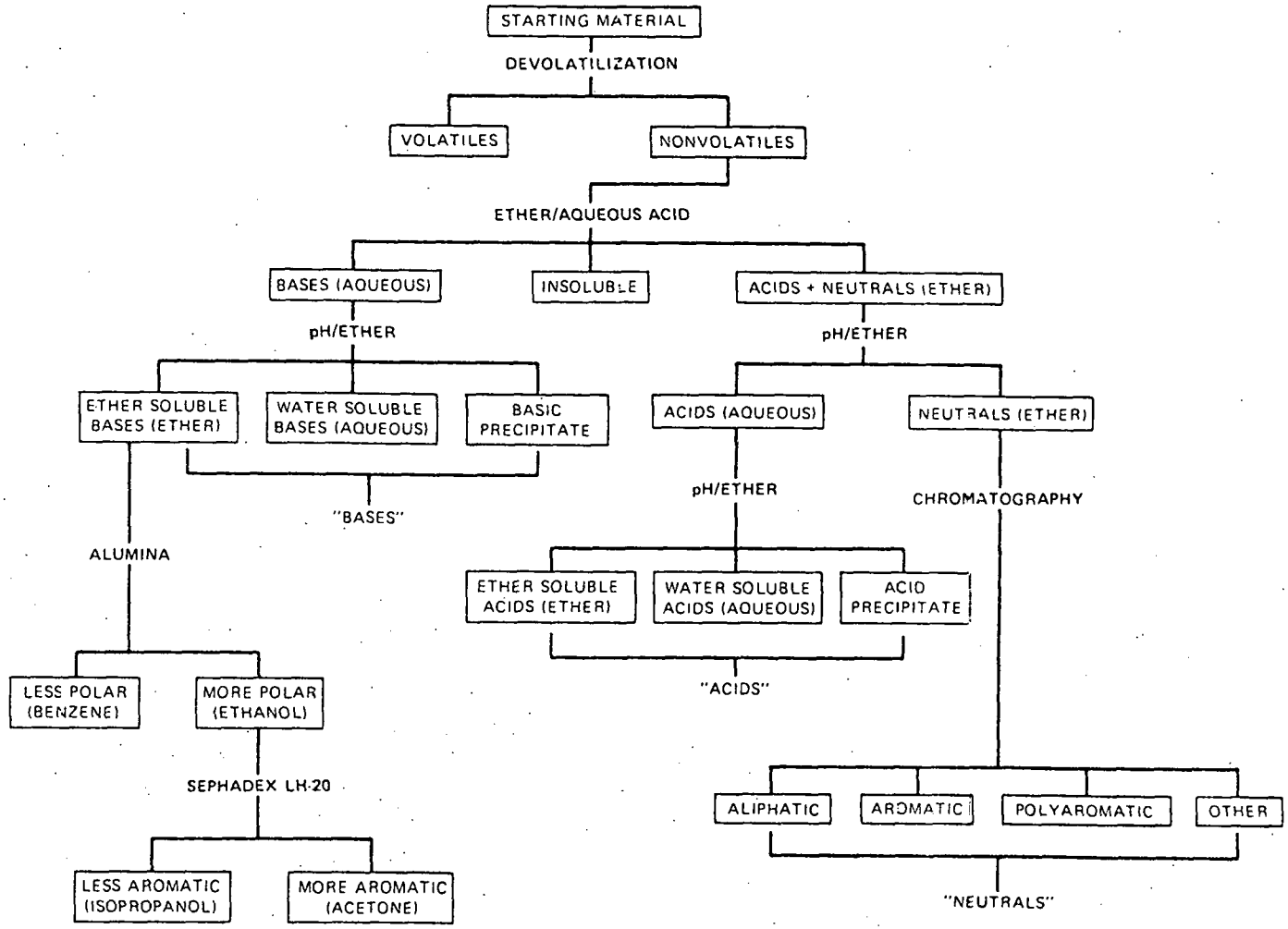
Peak No.	Compound	Peak No.	Compound
1	Trifluoroacetylimidazole	53	C_3-Aminonaphthalene
2	Aniline	54	Aminobiphenyl
3	C_1-Aniline	55	C_6-Aminonaphthalene
4	C_1-Aniline		C_1-Aminobiphenyl
5	C_1-Aniline	56	C_1-Aminobiphenyl,
6	C_2-Aniline		Aminofluorene
7	C_2-Aniline	57	C_1-Aminobiphenyl,
8	C_2-Aniline		Aminofluorene
9	C_2-Aniline	58	C_2-Aminobiphenyl
10	C_2-Aniline	59	C_2-Aminobiphenyl
11	C_2 Anilino	60	C_1-Aminobiphenyl
12	C_3-Aniline	61	C_2-Aminobiphenyl,
13	C_2-Aniline		Aminofluorene
14	C_3-Aniline	62	C_2-Aminobiphenyl,
15	C_3-Aniline		C_1-Aminofluorene
16	C_3-Aniline	63	C_1-Aminofluorene
17	C_3-Aniline	64	C_2-Aminofluorene
18	C_4-Aniline	65	C_2-Aminobiphenyl
19	C_4-Aniline	66	Aminoanthracene
20	C_4-Aniline	67	C_3-Aminobiphenyl
21	C_4-Aniline	68	Azapyrene
22	C_5-Aniline	69	Azapyrene
23	C_4-Aniline	70	C_3-Aminofluorene
24	Aminoindan	71	C_2-Aminofluorene
25	C_5-Aniline	72	C_4-Aminonaphthalene
26	C_1-Aminoindan	73	C_5-Aminoindan
27	C_5-Aniline	74	Aminoanthracene
28	C_2-Aminoindan	75	C_3-Aminobiphenyl,
29	C_2-Aminoindan		C_1-Aminoanthracene
30	C_1-Aminoindan	76	C_1-Azapyrene
31	C_2-Aminoindan,	77	C_1-Azapyrene
	Aminonaphthalene	78	C_1-Azapyrene
32	C_2-Aminoindan	79	C_5-Aminonaphthalene
33	C_2-Aminoindan		C_1-Aminoanthracene
34	C_1-Aminoindan	80	C_1-Aminoanthracene
35	C_2-Aminoindan	81	C_2-Aminoanthracene
36	Aminonaphthalene	82	C_2-Aminoanthracene,
37	C_3-Aminoindan		C_2-Azapyrene
38	C_2-Aminoindan	83	C_2-Azapyrene
39	C_1-Aminonaphthalene	84	C_2-Aminoanthracene,
40	C_1-Aminonaphthalene		C_2-Azapyrene
41	C_1-Aminonpphthalene	85	C_2-Aminoanthracene
42	C_3-Aminoindan	86	C_3-Azapyrene
43	C_3-Aminoindan,	87	Phenylnaphthylamine,
	C_1-Aminonaphthalene		Benzacridine
44	C_1-Aminonaphthalene	88	C_3-Aminoanthracene
45	C_2-Aminoindan	89	Phenylnaphthylamine
46	C_4-Aminoindan,	90	Aminopyrene
	C_2-Aminonaphthalene	91	C_1-Aminopyrene,
47	C_2-Aminonaphthalene		C_1-Benzacridine
48	Aminobiphenyl	92	C_1-Aminopyrene
49	Aminobiphenyl	93	C_1-Aminopyrene,
50	C_2-Aminonaphthalene		C_1-Benzacridine
51	C_4-Aminoindan	94	C_1-Benzacridine
52	C_2-Aminonaphthalene		

FIGURE CAPTIONS

Figure 1. Chemical Class Fractionation Procedure for Chemistry and Biotesting. [Material,] Step, (Phase)

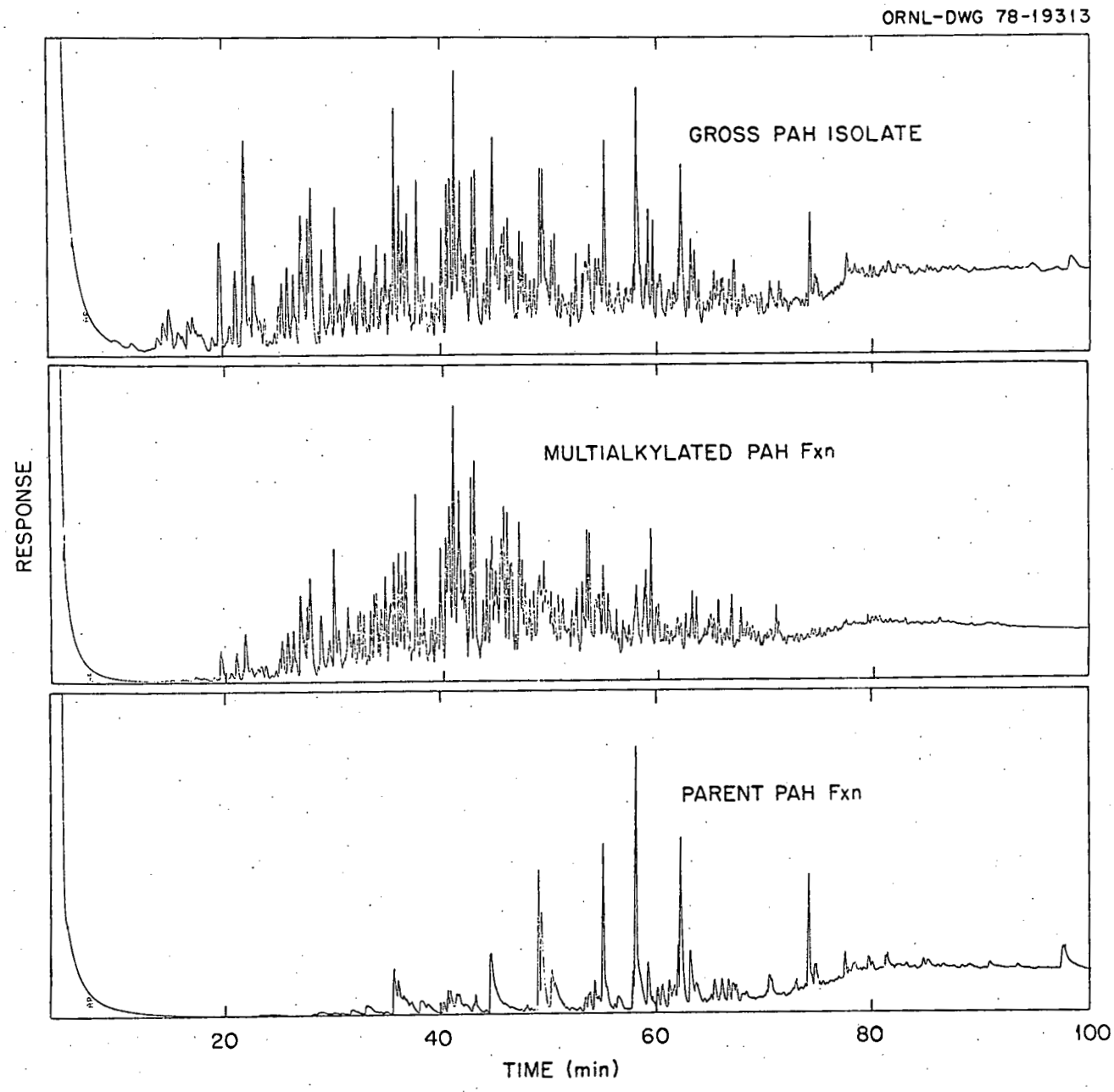
Figure 2. Gas Chromatograms of PAH and Multialkylated PAH Fractions

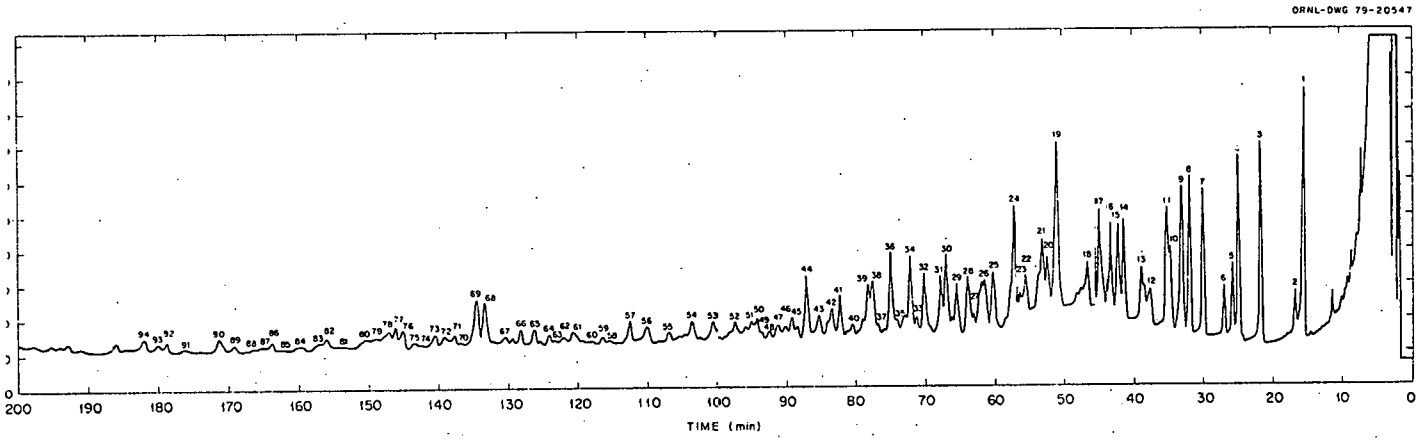
Figure 3. Gas Chromatogram of Trifluoroacetyl-Derivatized Acetone Subfraction of Synthoil No. 1202 ESB



CHEMICAL CLASS FRACTIONATION PROCEDURE FOR CHEMISTRY AND BIOTESTING.

MATERIAL, STEP, (PHASE)


ORNL-DWG 78-19313
GROSS PAH ISOLATE
MULTIALKYLATED PAH Fxn
PARENT PAH Fxn
RESPONSE
20
40
60
80
100
TIME (min)


ORNL-DWG 79-20547
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
TIME (min)