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Fractional Distillation as a Strategy for Reducing the Genotoxic Potential of SRC-II Coal Liquids: A Status Report

R. A. Pelroy
B. W. Wilson

Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Operated for the U.S. Department of Energy
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FRACTIONAL DISTILLATION AS A STRATEGY FOR
REDUCING THE GENOTOXIC POTENTIAL OF
SRC-II COAL LIQUIDS: A STATUS REPORT

R. A. Pelroy
B. W. Wilson

September 1981

Prepared for
the U.S. Department of Energy
under Contract DE-AC06-76RL0-1830

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GENOTOXIC POTENTIAL OF SRC-II COAL LIQUIDS:
A STATUS REPORT

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FOREWORD

This report presents results of studies on the effects of fractional distillation on the genotoxic potential of Solvent Refined Coal (SRC-II) liquids. SRC-II source materials and distilled liquids were provided by Pittsburg & Midway Coal Mining Co. Fractional distillations were conducted on products from the P-99 process development unit operating under conditions approximating those anticipated at the SRC-II demonstration facility. Distillation cuts were subjected to chemical fractionation, in vitro bioassay and initial chemical analysis. Findings are discussed as they relate to the temperatures at which various distillate cuts were produced. This document is the first of two status reports scheduled for 1981 describing these studies.

EXECUTIVE SUMMARY

In vitro biological testing and supporting chemical analyses have been performed on two sets of solvent refined coal (SRC-II) liquids which were fractionally distilled by different procedures. The first set of distilled samples comprised 18 boiling point cuts covering various temperature ranges between about 140° and 1050°F. Some cuts were as narrow as 10°F and of high precision. The second sample set consisted of three 50°F cuts between 700° and 850°F, together with the toppings (300° to 700°F) and the 850°F⁺ bottoms. All materials (parent liquids and distillate cuts) were screened for mutagenic activity against Salmonella typhimurium TA98, TA100, TA1537 and TA1535. Prophage induction in Escherichia coli (inductest) was also evaluated for all crude samples from the two sample sets to detect primary DNA damage. Initial chemical characterization, including fractionation and instrumental analysis by gas chromatography and mass spectrometry, was also performed on representative mutagenic and non-mutagenic distillate cuts.

In vitro biological data suggest that nearly all genetically active constituents in wide-boiling-range or full-boiling-range SRC-II liquids were fractionated into the higher-boiling range (i.e., above 700°F) constituents by distillation. For example, none of the crude liquid under 700°F boiling-point (b.p.) showed genetic activity. However, mutagenicity was observed between 700° and 750°F b.p. The transition from low to high mutagenicity occurred between 730° and 760°F. Above 750°F b.p., the specific mutagenic activity increased 2- to 4-fold reaching maximum values above 850°F b.p. DNA damage as detected by prophage induction occurred only in b.p. cuts above 850°F. Mutagenicity data (which appear to be a more sensitive indicator of genetic activity than prophage induction) indicated that constituents boiling below 700°F are genetically very weakly active (b.p. cuts just below 700°F) or inactive. Materials boiling below 700°F comprise about 80% of a wide-boiling-range, demonstration-facility-type SRC-II process distillate product. There was clear evidence that mutagens in the crude b.p. cuts representing the temperature range from just below 700°F to 800°F were not fully expressed. However, the degree of "masking" of genetic activity in

these materials was accounted for by chemical fractionation followed by bio-assay of the fractions. Even taking into account masking of genetic activity, the conclusion that nearly all genetic activity was contained in the above 700°F b.p. cuts appeared to be valid.

Initial chemical analysis of the b.p. cuts showed a general increase in the proportion of polar nitrogen-containing polycyclic compounds and polyaromatic hydrocarbons with increasing boiling point, while aliphatic hydrocarbon content decreased with increasing temperature. There was a general increase in compound heteroatom content and polarity as well as molecular weight as the boiling point of the cuts increased. High levels of mutagenicity were associated with fractions enriched in high-molecular-weight nitrogen-containing polyaromatic hydrocarbons (PAH). Mutagenicity was not associated with fractions enriched in neutral polyaromatic hydrocarbons. Nitrosation of nitrogen-containing fractions substantially reduced their mutagenicity suggesting that primary aromatic amines (PAA) were the main determinants of biological activity.

Biological and chemical data suggest that selective distillation of coal liquids during processing may be feasible to produce fuels which have lowered genotoxicity compared to the full-boiling range material.

IMPORTANT ABBREVIATIONS

BaP	= benzo[a]pyrene
b.p.	= boiling point
GC	= gas chromatography
GCMS	= gas chromatography mass spectrometry
HPLC	= high performance liquid chromatography
HRMS	= high resolution mass spectrometry
M/e	= mass to charge ratio
PAA	= polyaromatic primary amines
PAH	= polyaromatic hydrocarbons
Pfu	= Plaque forming units per petri plate
rev	= revertant colonies <u>Salmonella typhimurium</u> per petri plate in the Ames test
SRC-II	= Solvent Refined Coal Process II
b.p. X to Y°F	= constituents of set no. 1 or set no. 2 that boil between X and Y°F

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INTRODUCTION

As engineering designs for facilities to demonstrate direct coal liquefaction near completion, there is increased interest in possible process modifications which may be utilized to render demonstration products as safe as possible. Much of the current emphasis in this area concerns the potential genotoxicity which may result from worker or public exposure to high boiling coal liquids. Particular attention is being paid to possible mutagenic and carcinogenic effects. Two approaches to reduce genotoxic potential of high-boiling coal liquids through process modification have been investigated.

In the first, an SRC-II fuel blend was hydrotreated and the resulting materials were tested for mutagenicity and analyzed chemically. Results showed that hydrotreatment substantially altered the chemical makeup of the starting material and lowered its mutagenic activity.^(6,44) Similar results have been reported after hydrotreatment of shale oil and other coal liquids.⁽⁶⁾ Although it is outside the scope of this report to discuss the merits of commercial hydrotreatment, additional equipment and plant facilities would be required to accomplish this refining step, whether performed on-stream or post-production.

A common characteristic of various direct coal liquefaction processes studied to date is that genotoxic properties of products are generally found in high-boiling or heavy-end materials. For example, the SRC-II pilot plant yields three liquid streams designated light distillate (b.p. Amb -350°F) middle distillate (b.p. 350° to 550°F) and heavy distillate (b.p. 550° to 850°F), and although the two lower-boiling range SRC-II materials are acutely toxic in mammalian tests, microbial mutagenicity and mouse skin carcinogenicity are due almost exclusively to constituents in high-boiling distillates⁽²⁸⁾. These same characteristics have been observed in earlier work with other coal liquids.⁽⁴⁰⁾ Initial work in our laboratory showed that mutagenic activity of a SRC-II heavy distillate was found in a small portion of the total mass constituting the highest boiling fractions of the material.⁽⁴²⁾ This result suggested that mutagenic constituents in HD might be removed from the bulk material by simple distillation.

A chemical basis for fractionating genotoxic constituents into higher-boiling cuts by distillation can be rationalized by considering the relative

molecular weight and polarity of compounds recognized as carcinogens and/or mutagens in coal liquids. For example, all known or suspected mutagens and carcinogens identified in high-boiling coal distillates are polyaromatic. Some compound classes in this group include neutral polyaromatic hydrocarbons (PAH), polyaromatic azaarenes and carbazoles (containing a heterocyclic nitrogen), polyaromatic thiophenes (containing a heterocyclic sulfur), and polyaromatic primary aromatic amines (PAA). Less understood are bifunctional or multifunctional polyaromatic compounds which appear to be present and contain two or more heteroatoms. Almost all the above compounds have boiling points in excess of 250°C (490°F). Benzo[a]pyrene, for example, a PAH mutagen and suspected human carcinogen, would be expected to distill only in the highest boiling ranges (10% by weight) of the SRC-II heavy distillate. More polar hydroxylated PAH, polycyclic azaarenes, carbazoles, thiophenes, and in particular, the highly mutagenic PAAs would be expected to distill at even higher temperatures than the corresponding PAHs having comparable numbers of aromatic rings.

To test the hypothesis that genetic activity of SRC-II liquids could be reduced by distillation, a range of boiling point fractions (b.p. cuts) were produced under conditions simulating those expected at a demonstration facility. Process development unit (PDU) equipment was used, and some fractional distillates were produced to obtain quantitative data on weight distribution in various fractions. Each b.p. cut produced was tested for genetic activity. Biologically active cuts were chemically characterized by mass spectrometry and high resolution mass spectrometry and glass capillary gas chromatography. Some b.p. cuts were chemically fractionated, analyzed and subjected to additional bioassays.

This report describes initial mutagenicity testing, fractionation and chemical characterization of distillate cuts from a full-boiling range blend of SRC-II material produced with the P-99 PDU operated by Gulf Science and Technology Co. at Harmarville, PA. This one-ton-per-day unit was selected to produce the full-boiling range material because it can be operated to represent projected demonstration facility process conditions more closely than can the Fort Lewis pilot plant. However, these samples may not represent materials which may eventually be produced at a demonstration or commercial facility.

EXPERIMENTAL METHODS

SAMPLE MATERIALS

Two separate sets of fractionally distilled coal liquid samples were studied during the course of the work described here. Sample at number 1 consisted of 18 distillate cuts covering various boiling point (b.p.) ranges between 138° and 1055°F. Sample set number two consisted of distillate cuts ranging in temperature from 300°F to the 850°F+ bottoms. Between 700° and 850°F+ the cuts represented a 50°F b.p. range, and the weight percentages of the source material which distilled into each cut were recorded so that the cuts could be reblended in the proper ratio to yield wider distillate ranges in increments of 50°F.

The feedstocks to the distillation were obtained from the SRC-II processing of Powhatan No. 5 Mine coal on Process Development Unit P-99. No one product stream contained the entire boiling range of interest. Bottoms product from the debutanizer column was typically a heavy naphtha boiling range (146-519°F) material, while process solvent product boiled over the range 416-886°F and overlapped with the debutanizer bottoms boiling range. The only source of heavy distillate boiling above 900°F was atmospheric flash column bottoms, an internal process stream. Detailed description of the source materials and distillations along with physical, chemical and thermodynamic properties of the resultant materials is contained in reference 5.

OVERALL STRATEGY FOR FRACTIONATION, BIOTESTING AND CHEMICAL ANALYSIS OF DISTILLED SAMPLE MATERIALS

Figure 1 shows the approach for analysis of the 18 distillate cuts of Set no. 1 and 5 distillate cuts for Set no. 2.

Sample set no. 1 materials arrived at PNL in July 1980. Set no. 2 materials arrived in February 1981. High performance liquid chromatographic (HPLC) separations were employed to enrich certain compound classes in specific fractions which could then be bioassayed. This approach helps to identify genotoxicants which, although present, may be in such low concentrations that they are not expressed in the crude b.p. cuts. Ames testing was conducted to identify point and frameshift mutagens. Prophage induction was used to assess primary DNA damage. Low resolution probe inlet mass spectrometry (MS) and/or gas chromatography mass spectrometry (GCMS) were used on the remaining crude b.p. cuts and on certain HPLC fractions derived from these materials. Selected data from our analyses will be reported. Results from high resolution mass spectrometry (HRMS), GCMS and probe inlet MS analyses of sample set no. 1 and no. 2 materials will be described in a future report.

FIGURE 1. Flow Sheet for Sample Work-Up and Analysis

SAMPLE FRACTIONATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Each distillate sample from set no. 1 was fractionated by HPLC using a semi-preparative scale (10 mm x 250 mm), normal phase -NH₂ column. A continuous ternary gradient elution scheme was used in which samples were eluted sequentially with hexane, methylene chloride, and isopropanol. This elution sequence separates synthetic fuel materials into four distinct regions designated as non-polar (Region I), moderately polar (Region II), transition (Region III), and strongly polar (Region IV). The separation pattern allows collection of fractions (Figure 2) which are significantly different in their chemical class composition. Region I elutes with hexane and contains hydrocarbons, PAHs, and some non-polar heterocycles. The moderately polar region, Region II, elutes with increasing methylene chloride concentration and contains nitrogen heterocycles, PAA, azaarenes, carbazoles, and alkylated and partially

FIGURE 2. Typical HPLC Chromatogram of a B.P. Cut Showing Major Polarity Regions Described in Text. This chromatogram shows b.p. (639-689°F) from sample set no. 1. Elution times for various regions are indicated. Detection was by uv absorption at 250 nm.

hydrogenated counterparts of these materials. Highly polar compounds elute in Region IV with increasing proportions of isopropane in methylene chloride. Region IV contains phenols and hydroxylated and multifunctional PAH and heterocycles. The transition region (Region III) has not yet been well characterized but may contain bifunctional aromatic compounds such as hydroxylated PAA.

Ternary gradient HPLC separation provides considerable separation of constituents based on compound class. Eluants from this separation process were collected in discrete fractions, dried, weighed, and prepared for further chemical and microbial mutagenicity analysis.

FRACTIONATION BY CHEMICAL CLASS SEPARATION AT AMBIENT PRESSURE

The second set of 50°F b.p. cut samples was fractionated by a scheme recently developed by Later et al. at Brigham Young University under subcontract to PNL.⁽¹²⁾ The procedure yields aliphatic, neutral PAH, phenol and nitrogen PAH fractions. The latter fraction is further divided into azaarene, carbazole and PAA subfractions which are essentially functional group specific. Application of this procedure (Figure 3) allows a rapid estimate of relative amounts of the first four compound types in the 50°F⁺ cuts.

As discussed in reference 12, a consistent nomenclature system for polycyclic aromatic compounds has recently been proposed. This nomenclature will eventually be adopted by this laboratory for work in the fossil fuel area. The original publication from which the chemical class method was adopted utilized this nomenclature and hence it is used in the diagram of Figure 3.

PAC refers to the polycyclic aromatic compounds in general, including both hydrocarbons and heterocyclic compounds, while PAH refers only to the polycyclic aromatic hydrocarbons. PANH refers to the polycyclic aromatic nitrogen heterocycles, in which the nitrogen heteroatom is part of the ring structure. Therefore, 2°-PANH represents PANH in which the nitrogen heteroatom is part of a 5-membered ring and has a hydrogen atom covalently attached (i.e., indole, carbazole), while 3°-PANH represents PANH in which the nitrogen heteroatom is part of a 6-membered ring (i.e., acridine). APAH refers to PAH which have an amino group attached to a carbon atom in the ring. This amino group can be

FIGURE 3. Flowsheet for Chemical Class Fractionation According to Method of Later et al.⁽¹¹⁾

either primary, secondary, or tertiary, and hence resulting compounds are designated as 1°-, 2°-, or 3°-APAH. N-PAC refers collectively to all PAC which have a nitrogen heteroatom.

CHEMICAL ANALYSIS

The chemical analyses described here were carried out in support of biological testing and are in no way intended as a substitute for accepted API or ASTM methods for the analysis of hydrocarbon fuels.

Gas Chromatography

Hewlett Packard 5840A or 5880 GCs equipped with flame ionization and nitrogen-specific detectors were used to analyze selected crude cuts and nitrogen-rich chemical fractions. Results from simultaneous two-column injection onto identical SE52 fused silica columns, one connected to the nitrogen-specific detector, and the other to the FID, allowed estimation of the proportion of gas chromatographable compounds in a complex mixture containing nitrogen. GC was used extensively to characterize distillate cuts and fractions resulting from the various chemical separations. However, due to their complexity and the narrow temperature range over which they eluted from the column, GCMS was required for specific identifications in most intact distillate cuts and HPLC fractions.

Gas Chromatographic-Mass Spectrometry

GCMS analyses were performed on a VG Micromass ZAB 1F mass spectrometer interfaced to a Hewlett Packard 5840A GC. The GC column was 0.25 mm ID fused silica coated with SE54 liquid phase and used in the splitless injection mode. The column end was inserted in a quartz glass tube to within 2 mm of the ion source. The quartz tube was fitted tightly against the ion source to prevent diffusion or leakage of the column eluate. Scans were taken at rates of between 2 and 5 seconds per decade with nominal resolution of 1000. Perfluor-alkane was used for calibration and all spectra were acquired and stored on a PDP8A based VG 2035 data system.

High Resolution Mass Spectrometry

In analyzing higher molecular weight fractions from fossil fuels, and especially synfuels, GC has serious limitations. As a rule, polar compounds having more than 4 rings will not be transported through the GC column due to their low volatility.

Probe inlet mass spectrometry, while not yielding the valuable retention time data available by GC inlet, does not have the same restrictions relative to molecular weight and polarity as GCMS. In particular, when probe inlet mass spectra are acquired at sufficient resolution, the elemental compositions of many (if not most) ion peaks present may be determined.

Highly aromatic synthetic crudes produced from coal are especially amenable to HRMS analysis since aromatic molecular ions tend to be stable and relatively intense in the mass spectra of the complex mixtures investigated here.

HRMS analyses were performed on a VG Micromass ZAB 1F double focussing mass spectrometer operated at 8 kV. Unless otherwise stated, nominal resolution was adjusted to 20,000 $M/\Delta M$ (10% valley) and high boiling perfluoro-alkane was used as an internal standard. The mass spectrum was scanned at a rate of 30 sec per decade, and spectra acquired were mass calibrated and stored on a PDP8A based VG 2235 data system. The same system was used at low mass resolution to obtain approximate estimates of the overall molecular weight range of fractions from the second sample set.

Low Resolution Probe Inlet Mass Spectrometry

LRMS analyses were also carried out on the ZAB 1F spectrometer described above at 6 kV accelerating voltage. The method provided rapid characterization of b.p. cuts or cut fractions not amenable to GC. Sample introduction was via heated probe inlet and the sample was eluted between a nominal 100° and 400°C. Spectra were recorded between mass 500 and mass 20 at 5 sec per decade. Nominal resolution was adjusted to 1000 $\Delta M/M$ for these analyses. Probe inlet MS provides the same advantages as HRMS compared to GCMS. However, elemental composition information is not available.

Chemical Derivatization

Silanization procedures were performed on some samples to demonstrate the presence of primary amino or hydroxyl functionalities. Samples to be derivatized were treated with 3 to 4 volumes of Tri Sil TBT® and warmed at 60°C for 5 to 10 min. Additionally, several more specific techniques for differentiating between non-gas-chromatographable azaarenes and PAA were developed. These include treatment with nitrous acid, derivitization with trifluoroacetic acid or pentafluoropropionic anhydride after chemical separation of amines from carbazoles and azaarenes. The latter method, along with the distributions of some 60 different primary aromatic amine compounds in 50° boiling fractions of

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SRCI process solvent, is described in reference 47. Representative selected ion chromatograms resulting from the application of this method to a 700° to 850°F blend of sample set no. 1 are discussed on page 52 of this report.

MUTAGENESIS ASSAYS

Agar plate mutagenicity assays were performed as described by Ames et al.⁽¹⁾ using four Salmonella test strains: Salmonella typhimurium TA98, TA100, TA1535 and TA1537. The mutagen phenotype defining these strains was determined in each experiment (Table 1).

Dimethylsulfoxide (DMSO) was used as a solvent. In addition to the mutagens (Table 1) used to check the phenotypes of Ames Salmonella strains, fixed concentrations of 2-aminoanthracene, benzo[a]pyrene and a complex basic fraction from SRC-II heavy distillate were tested against the rat liver (S9) homogenates used for metabolic activation. Optimum levels for S9 were established and monitored daily during the course of our experiments. Negative controls included the Ames/Salmonella typhimurium test strains, plus and minus microsome enzymes (S9), and appropriate solvent controls. Revertant (rev) colonies per petri plate were counted electronically using a Biotran II automated colony counter (New Brunswick Scientific Co., Inc., Edison, NJ).

TABLE 1. Mutagen Phenotype of Ames Tester Strains Used to Bioassay SRC Materials

<u>Compound</u>	<u>µg/Plate</u>	<u>S9</u>	Response for Ames Test Strain:			
			<u>TA98</u>	<u>TA100</u>	<u>TA1535</u>	<u>TA1537</u>
2-Aminoanthracene	1	+	(+)	(+)	(+)	(+)
9-Aminoacridine	50	+	(-)	(-)	(-)	(+)
Nitrosoguanidine	1	-	(-)	(+)	(+)	(-)
Dimethylbenzacridine	10	+	(-)	(+)	(-)	(-)

+ indicates a response in revertant colonies at least 2 times above background.

- indicates less than a twofold response above background.

The crude boiling point cuts were assayed for mutagenicity at concentrations of 2, 4, 8, 10, 20 and 50 μg or 0.2, 0.4, 0.8, 1.0, and 2.0 μg per petri plate. Each concentration was assayed in duplicate. Stock solutions of SRC-II distillate cuts were prepared at concentrations of 10,000 $\mu\text{g}/\text{ml}$ in DMSO and stored at -80°C when not in use.

Fractions prepared from crude b.p. cuts by HPLC were assayed mostly in the same concentration ranges described for crudes; i.e., 2 to 50 μg or 0.2 to 5.0 μg . HPLC fractions were stored at -80°C in DMSO under a nitrogen atmosphere.

Specific mutagenic activity of b.p. cuts or chemical fractions was expressed as revertant colonies of S. typhimurium TA98/ μg of test material, estimated by linear regression analysis of dose-response data. A positive test was one having a maximum mutagenic response in rev colonies twofold or greater above background, and a correlation coefficient 0.8 or above. To estimate recovery of genetic activity, Ames experiments were replicated several times, depending on the experiment, to obtain an average value for mutagenic potency in rev/ μg . The average value was then multiplied by the weight percent (wt%) of a given fraction to yield a weighted mutagenic activity in relation to the crude b.p. cut or fraction.

Nitrous Acid Experiments

Nitrosation experiments were conducted using a modification of the procedure described by Yoshida and Matsumoto.⁽⁴⁹⁾ Sodium nitrite solution was prepared by dissolving 600 mg of the salt in 30 ml 0.3 HCl (v/v). A 0.3% hydrochloric acid solution was used as a control on the acidic nitrite solution. Both acidic solutions were filter-sterilized prior to use. To begin nitrosation experiments, 100 to 1500 μg of a given pure chemical or 100 to 5000 μg of a given complex chemical mixture were added to 2 ml of acidic nitrite solution or the acid solution without nitrite. The complex fractions or pure chemicals were also added to 2 ml DMSO, the solvent usually used for introducing mutagens in the Ames/Salmonella assay. Both the acidic and DMSO mixtures were mixed vigorously and allowed to stand 90 min at room temperature (about 25°C). From 5 to 50 μl of acid or DMSO mixture were removed and added

directly to the 45°C liquid top-agar already containing the other components of the Ames test minus the activating enzymes. Activating enzymes contained in the hepatic, Aroclor (1254) induced (see below), crude microsomal (S9) homogenates⁽¹⁾ were added last. Contents of the top-agars were then poured on the agar plates as described above. The pH of top-agar varied from 6.6 to 7.2, depending on the volume of acidic solution added. This range of pH values did not affect sensitivity of the assay.

Prophage Induction (Inductest)

Prophage induction was measured using Escherichia coli K12 strain (8177). This organism is lysogenic for a special form of the bacterial virus lambda which is induced to undergo a lytic cycle in response to primary DNA damage.⁽¹⁵⁾ Phage produced by the lytic cycle were detected by plaque formation with E. coli K12 (6340) which is sensitive to the phage harbored by E. coli (8177). About 10^8 target cells of E. coli (8177) were exposed overnight (16 hr) to a chemical or (SRC-II) complex mixture. Metabolic activation was provided by S9 homogenates, as described above for the Ames assay.

The mixture containing exposed cells and induced prophage were then filtered through a 0.45 micron Millipore to separate phage from uninduced target cells. Filter-sterilized prophage suspensions were diluted serially and assayed for plaque formation using E. coli (6340) as described elsewhere.⁽¹⁵⁾

Plaques were counted at 16 to 24 hr with an automated colony counter, as described for the Ames assay. Locations of plaques were also marked on the back of petri plates with a marking pen and counted with surface illumination. A positive test had a response over background ratio of two or greater.

Preparation of Liver (S9) Homogenates

Induction of mutagen activating enzymes was achieved with Aroclor 1254 (Monsanto Chemical Corp.), 250 mg/kg body weight, injected intraperitoneally into male Sprague-Dawley rats. Rats were sacrificed on the fourth day after injection. After sacrifice, livers were perfused with ice-cold 0.154 M KCl and removed for preparation of S9 homogenates, following standard procedures.⁽¹⁾

RESULTS

ANALYSIS OF BOILING POINT CUTS BY MICROBIAL MUTAGENESIS AND PROPHAGE INDUCTION ASSAYS

Crude b.p. cuts from sample set no. 1 (138° to 1055°F), set no. 2 (300° to 850°F⁺), and HPLC fractions from set 1 were tested for genetic activity in two microbial assay systems. Mutagenesis was measured with the *Salmonella* histidine reversion (Ames) standard plate incorporation test,⁽¹⁾ and with a variant of the Ames test based on pre-incubation of target cells in liquid before plating.⁽⁴⁷⁾ Prophage induction with a special lysogenic strain of *Escherichia coli* (inductest) was also carried out. The inductest was employed to detect primary DNA damage induced by the b.p. cuts.

Sample set no. 1 consisted of 18 b.p. cuts (Table 2, Figure 4). Temperature ranges for b.p. cuts of sample set no. 1 below 700°C were mostly less than 50°F. Temperature intervals of the higher b.p. cuts (i.e., above 700°F) were more closely spaced up to 761°F (b.p. 739° to 761°F) except for one wide-ranging b.p. (705° to 825°F). The three highest-boiling cuts (782° to 1055°F) were spaced at intervals of about 100°F.

None of the b.p. cuts with an upper temperature limit below 582°F were genetically active (Table 2, Figure 4). A questionable mutagenic response for b.p. 639° to 689°F was observed for *S. typhimurium* TA98 using the modified Ames test. However, no activity was detected for this b.p. cut with the standard Ames test and the four Ames tester strains TA98, TA100, TA1535 and TA1537. The inductest was also negative, confirming results of the Ames tests for the 10 b.p. cuts covering the temperature range from 138° to 582°F.

In contrast, b.p. cuts above 700°F were all mutagenic in both standard and modified Ames assays. Three strains that respond to frameshift mutagens *S. typhimurium* TA98, TA100 and TA1537 were mutagenized by b.p. cuts covering the temperature interval 700° to 1055°F. *S. typhimurium* TA1535 which responds only to point mutagens was not mutagenized by any b.p. cuts except for a weak response for b.p. 914° to 1055°F. These data confirm previous work with high-boiling SRC-I and -II distillates^(2,20,24) and other synthetic fuels,^(4,21,23,27)

TABLE 2. Genetic Activity of Crude B.P. Cuts from Sample Set No. 1 Derived from the SRC-II Process Under Conditions Approximating a Demonstration Facility

B.P. Cut of Sample Set 1	Mutation						DNA Damage Inductest(c)
	TA98(a)	TA100(a)	TA1535(a)	TA1537(a)	TA98(b)	TA100(b)	
(138° - 160°F)	0	0	0	0	0	0	
(202° - 224°F)	0	0	0	0	0	0	0.9
(248° - 257°F)	0	0	0	0	0	0	1.8
(269° - 288°F)	0	0	0	0	0	0	1.4
(320° - 341°F)	0	0	0	0	0	0	1.5
(369° - 381°F)	0	0	0	0	0	0	1.7
(424° - 437°F)	0	0	0	0	0	0	1.1
(477° - 488°F)	0	0	0	0	0	0	1.1
(522° - 534°F)	0	0	0	0	0	0	0.9
(565° - 582°F)	0	0	0	0	0	0	1.10, 0.73
(639° - 689°F)	0	0	0	0	0.64	0	0.73
(705° - 825°F)	4.87 ± 2.25	2.93 ± 1.65	0	1.36	--	--	1.8
(710° - 730°F)	0.53 ± 0.42	1.76 ± 1.51	0	0	--	--	1.3
(739° - 761°F)	30.0 ± 10.9	2.15 ± 0.18	0	0.62	--	--	2.0
(645° - 782°F)	14.6 ± 3.7	3.48 ± 0.66	0	0.28	--	--	1.4
(782° - 878°F)	77.4 ± 21.8	3.31 ± 2.44	0	1.78	--	--	2.4
(867° - 967°F)	98.6 ± 22.8	5.40 ± 3.68	0	3.26	68.53	3.18	3.5
(914° - 1055°F)	85.0 ± 19.4	4.82 ± 1.74	0.11	8.00			4.5

(a) Specific mutagenic activity in rev/μg from standard Ames plate incorporation test with S. typhimurium as the target cell. Average of 3 experiments ± SE.

(b) Specific mutagenic activity in rev/μg from pre-incubation Ames test with S. typhimurium TA98 or TA100 as target organisms. Single experiment for each strain.

(c) Inductest expressed in ratio of Pfu for exposed cells to Pfu for unexposed cells. A positive test defined as 2-fold increase in ratio of Pfu exposed/Pfu unexposed.

FIGURE 4. Genetic Activity for B.P. Cuts of Sample Set No. 1. Ames mutagenicity data is from Table 5 for *S. typhimurium* TA98 in the standard plate incorporation test. DNA damage estimated by the inductest is also from Table 5. Note difference in scale of ordinates.

and suggest that mutagens in these materials are the frameshift type. *S. typhimurium* TA98 was the most responsive Ames strain to mutagenesis by the higher-boiling point cuts of sample set no. 1.

In quantitative terms, mutagenicity of b.p. 710° to 730°F was comparatively low. However, there was a sharp increase in activity (ca 60-fold) of b.p. 739° to 761°F. Approximately another 1.5-fold increase in specific mutagenic activity occurred in the highest-boiling b.p. cuts, ranging from 782° to 1055°F. These data suggest a large increase in the concentration of mutagens in materials boiling between 730° and 760°F of set no. 1.

The inductest was positive only for the three highest b.p. cuts from 782° to 1055°F. Thus, prophage induction was a less sensitive indicator of genetic damage induced by the chemically complex b.p. cuts than the Ames assays.

It is noteworthy that mutagenesis results from pre-incubation tests agreed with those obtained with the standard Ames test for all b.p. cuts of set no. 1.

Additionally, the pre-incubation test appears to be more sensitive than the standard Ames assay for polycyclic mutagens similar to those known or suspected to be constituents of the SRC-II heavy-end materials.^(7,19,22,30,31,32,41,43) However, the pre-incubation test does not appear to be more sensitive to mutagens in b.p. cuts from sample set no. 1 than the standard Ames assay. With one possible exception, the pre-incubation Ames test did not reveal mutagens not detectable in the standard plate incorporation test.

All genotoxins in b.p. cuts expressed in the Ames assay or inductest required metabolic activation. This finding is in agreement with previous work on SRC-II high-boiling liquids,^(2,20,24) various SRC-I materials^(2,24) and other coal liquids.⁽⁴⁾ Assays for direct-acting mutagens in the b.p. cuts of Table 2 were all negative (data not shown).

Results described in Table 2 were confirmed with a second set of b.p. cuts from a wide-boiling range process solvent obtained from the Harmarville pilot plant (Table 1). Lower boiling constituents in this second set were contained in a single cut, b.p. 300° to 700°F. Three cuts, b.p. 700° to 750°F, 750° to 800°F, and 800° to 880°F were distilled at 50°F intervals. The highest boiling constituents were contained in b.p. 850°F⁺. Mutagenesis was determined with S. typhimurium TA98 and TA100. The inductest was again used to detect primary DNA damage.

Results for sample set no. 2 were similar to those obtained for the first sample set. First, b.p. 300° to 700°F was not active in either the Ames assay or inductest. The three 50°F b.p. cuts (700° to 850°F) were mutagenically active against two Salmonella strains. S. typhimurium TA98 responded with about equal sensitivity in both the standard Ames plate incorporation and pre-incubation tests. S. typhimurium TA100 appeared much more responsive to mutagenesis by the above 700°F b.p. cuts of sample set no. 2 than to the comparable cuts from sample set no. 1. The inductest was positive only for b.p. 850°F⁺ of sample set no. 2. In quantitative terms, data for both sample sets were comparable with respect to mutagenesis of S. typhimurium TA98 and prophage induction, showing a sharp increase in specific mutagenic activity for b.p. cuts in the 800° to 850°F⁺ temperature range. The in vitro bioassay data of Table 3 are summarized in Figure 5.

TABLE 3. Genetic Activity of B.P. Cuts of Sample Set No. 2 From Set No. 1, Produced Under Conditions Designed to Approximate Those of a SRC-II Demonstration Facility

Sample Designation (Set No. 2)	Mutation			DNA Damage Inductest ^(c)
	TA98 ^(a)	TA98 ^(b)	TA100 ^(b)	
b.p. (300 - 700°F)	0	0	0	1.3
b.p. (700 - 750°F)	28.9 ± 11.7	16.7	3.2	0.8
b.p. (750 - 800°F)	35.8 ± 19.7	28.6	5.5	1.2
b.p. (800 - 850°F)	123.8 ± 19.1	138.8	71.0	1.8
b.p. (850°F ⁺)	88.8 ± 22.7	69.5	48.4	6.2

- (a) Specific mutagenic activity in rev/μg from standard Ames plate incorporation test with S. typhimurium TA98 as the target cell. Average of 3 experiments ± SE.
- (b) Specific mutagenic activity in rev/μg from pre-incubation Ames test with S. typhimurium TA98 or TA100 as target organisms. Single experiment for each strain.
- (c) Inductest expressed in ratio of Pfu for exposed cells to Pfu for unexposed cells. A positive test defined as 2-fold increase in ratio of Pfu exposed/Pfu unexposed.

EXPRESSION OF MUTAGENICITY: DETERMINANT MUTAGENS

Presence or absence of genetically active agents in complex mixtures cannot be determined solely on the basis of bioassay or chemical data alone. As a first approximation, it is often assumed that bioassay activity is proportional to the concentration of the active agent(s) responsible for the observed effect. (4,10,11,37,38) However, absence of a genetic effect for a complex chemical mixture does not necessarily imply absence of genotoxins, because: 1) genetic activity may have been masked due to composition, or 2) bioassay may have been inappropriate or insensitive for the mixture in question.

The distribution of genetic activity in b.p. cuts from the two sample sets suggests that agents responsible for mutagenicity in the Ames test and prophage induction were concentrated entirely in the nominal 15 weight percent of both sample sets boiling above 700°F. Concentrations of mutagens in these b.p. cuts appeared to increase slowly with temperature between 700° to 800°F, and then rapidly above 800°F. Thus, based on genetic activity alone, it would appear

FIGURE 5. Summary of Genetic Activity for B.P. Cuts of Sample Set No. 2 Produced Under SRC-II Demonstration Facility Conditions. Ames mutagenicity data (from Table 3) is for *S. typhimurium* TA98 in the standard plate incorporation test. Inductest data used to estimate primary DNA damage also from Table 3. Note difference in scale on ordinates.

that mutagens or prophage-inducing materials were absent in fractions from both sample sets boiling below 700°F. It is important to establish with all possible certainty that lack of genetic activity in the b.p. cuts indicates that genotoxins are absent or in minimal concentrations.

One approach towards this end is chemical fractionation and analysis, followed by additional bioassay. As described below, selected crude b.p. cuts were analyzed in this way so that mutagens that might be present in the under 700°F b.p. cuts could be concentrated and detected. Additionally, we wished to determine if total mutagenic activity in HPLC fractions from the above 700°F

b.p. cuts (i.e., mutagenically active b.p. cuts) was equal to that of crude unfractionated materials. Significant masking of mutagenicity due to composition of the crudes might be detected by recoveries of activity in excess of those expected for crudes. Finally, the HPLC fractions were nitrosated to evaluate the importance of PAA in the mutagenicity of b.p. cuts. Previous work showed that significant losses of mutagenic activity after nitrosation of SRC-II liquids was diagnostic for PAA⁽²⁵⁾ as had been previously demonstrated for PAA in pyrolyzed foods.⁽⁴⁸⁾

A second approach involved blending the b.p. cuts in the proportions which they were found in prior to fractional distillation. The "reblended" cuts were then bioassayed to estimate genetic activity of a given b.p. cut (or cuts) in the complex mixture. If a blend was substantially lower in genetic activity than expected from summing the weighted specific activities of its parts, expression of mutagenicity (or genotoxicity) may have been inhibited by chemical composition of the mixture. Conversely, higher-than-expected genetic activity would suggest that total composition of the whole material is capable of exerting a synergistic effect, enhancing mutagenic (genotoxic) activity of the complex mixture. Of the two, suppression of mutagenic activity would be more damaging when evaluating a complex mixture for genotoxins with in vitro assays.

Mutagenicity of Reblended B.P. Cuts

Set no. 2 was chosen because it consisted of contiguous (non-overlapping) b.p. cuts whose relative proportions or wt% of whole set no. 2 were known (Table 4, Figure 6). Experiments were conducted as follows: individual b.p. cuts (not blended) were assayed for mutagenicity and their specific mutagenic activities were determined. The wt% of these fractions was then used to calculate a weighted activity based on the expected contribution of each cut to the whole material. The basic assumption was that mutagenic activities in whole material were additive; i.e., equal to the sum of the various b.p. cuts that comprise the material.

The b.p. cuts were also blended in the proportion they occur in set no. 2, and assayed for mutagenicity. Each blend contained the major component of set no. 2, b.p. 300° to 750°F plus one or more of the other cuts. The other

TABLE 4. Mutagenic Activity of B.P. Cuts and Reblended B.P. Cuts from a SRC-II Process Solvent Produced Under Demonstration Facility Conditions

B.P. Cut or Blend of Sample Set No. 2	Measured Specific Activity ^(a) (rev/ μ g)	Wt% of Whole HPS ^(b)	Calculated Weighted Specific Activity ^(c) (rev/ μ g)	Ratio Calculated/ to Measured
<u>B.P. Cuts</u>				
(300° - 700°F)	0	78.9	0	--
(700° - 750°F)	42.43 $\pm$ 7.27	5.5	2.33	--
(750° - 800°F)	60.87 $\pm$ 7.38	7.4	4.50	--
(800° - 850°F)	68.85 $\pm$ 23.92	3.5	2.41	--
(850°F ⁺)	92.99 $\pm$ 10.20	4.7	4.37	--
<u>B.P. Blends</u>				
(300° - 750°F)	5.24 $\pm$ 0.66	84.4	2.76	0.53
(300° - 800°F)	8.87 $\pm$ 3.43	91.8	7.44	0.84
(300° - 850°F)	12.34 $\pm$ 2.74	95.3	9.70	0.79
(300° - 850°F ⁺)	17.09 $\pm$ 4.32	100.0	13.61	0.80

(a) Determined by the standard Ames plate incorporation test with S. typhimurium TA98 as the target cell. Average of 5 experiments $\pm$ SE.

(b) Fraction of the given b.p. cut or blend in whole HPS x 100.

(c) For the b.p. cuts, weighted specific activities were calculated (relative to whole HPS) by multiplying (wt%/100) for a given cut x specific mutagenic activity of that cut to yield its expected contribution to the specific mutagenic activity of whole HPS.

For the blends, the weighted specific mutagenic activities of the individual b.p. cuts were summed to give the expected values relative to whole HPS, and this value divided by (wt%/100) of the blend; i.e., to give the activity of the mixture taking into account that fraction of whole HPS not contained in the blend.

FIGURE 6. Summary of Mutagenicity Data for Reblending Experiment. Measured values are from Ames data on blended samples. Calculated values were obtained from the wt% of a given blend in unfractionated source material and the specific mutagenic activities of the individual b.p. cuts in that blend.

four b.p. cuts were added stepwise to yield mixtures equivalent to b.p. cuts of 300° to 750°F, 300° to 800°F, 300° to 850°F and 300° to 850°F⁺, respectively. Both the unblended and rebended cuts were assayed in replicate experiments and calculated (expected) mutagenic activities were compared with observed activities (Table 4).

The individual (unblended) b.p. cuts had specific mutagenic activities similar to those observed in the first set of Ames assays with sample set no. 2 (see Table 3). The unblended cuts differed slightly in that they showed more mutagenic activity in the temperature range 700° to 800°F, and less activity between 800° to 900°F⁺ than in the previous experiments. From these measured activities of the unblended b.p. cuts, weighted specific mutagenic activities were calculated to estimate specific mutagenic activity in the rebended cuts.

Measured specific mutagenic activity of the blends was generally close to calculated values (Table 4). For the three blends representing b.p. 300° to 800°F, 300° to 850°F and 300° to 850°F⁺, calculated values were about 80% of observed values. For b.p. 300° to 750°F, the observed value was about twice the calculated value. These data are shown graphically in Figure 3.

Thus, the mutagenically inactive crude b.p. 300° to 700°F appeared to exert a slight synergistic effect on mutagenicity of the other b.p. cuts. Enhancement was about 2-fold for b.p. 700° to 750°F but much less for the other three b.p. cuts. Except for the blend representing b.p. 300° to 750°F, enhancement was minimal. The fairly close agreement between observed and calculated mutagenic activities suggests that genetic effects in the blended mixtures were approximately independent and additive. Thus, the major portion of HPS (i.e., the b.p. 300° to 700°F) is unlikely to inhibit expression of genetic activity in the higher-boiling cuts, or mutagens that might be contained in b.p. 300° to 700°F.

Bioassay of HPLC Fractions

Several b.p. cuts of sample set no. 1 were closely spaced in the temperature range 700° to 750°F. This temperature range is where b.p. cuts from the two sample sets begin to show mutagenicity in the Ames assay system. Four b.p. cuts from sample set no. 1 (two representing the temperature range just below 700°F, one in the transition range where mutagenicity is first expressed, and one representing a temperature range of maximum mutagenicity) were selected for chemical fractionation, nitrosation, and bioassay.

Data from these experiments are shown in Tables 5 through 8, and summarized in Figures 7 to 11. HPLC separated b.p. constituents into four regions characterized by compounds of increasing polarity (Figure 2). Neutral compounds, including highly uv absorbing PAHs, were concentrated in Region I. Moderately polar constituents, enriched in compounds such as PAA, azaarenes, carbazoles and phenanthrolines eluted in an envelope of strongly uv absorbing peaks in Region II. Constituents of Region III are less well characterized, but appear to contain bifunctional polycyclics of moderate to high polarity. Region IV was enriched in polyaromatic phenolic compounds that absorbed strongly in the uv region.

TABLE 5. Mutagenicity of HPLC Fractions from B.P. 639 to 689°F Cut of Sample Set No. 1 Before and After Treatment with Nitrous Acid

Fraction	Wt (μg)	Wt%	Specific Mutagenic Activity (rev/μg) ^(a)		Weighted Specific Mutagenic Activity (rev/μg) ^(b)	
			Untreated	Nitrous Acid	Untreated	Nitrous Acid
1	64,110	69	0	0.27	0	0.19
2	1,850	2	0	0	0	0
3	2,820	3	0	0	0	0
4	5,390	6	5.73	0	1.34	0
5	2,410	3	34.29	152.00	1.03	4.56
6	4,380	5	0.80	108.95	0.04	5.45
7	2,450	3	0	6.82	0	0.20
8	3,180	3	0	0.57	0	0.02
9	3,980	4	0	0	0	0
10	1,470	2	0	0	0	0
11	270	<1	0	0	0	0
TOTALS	92,310	100			TOTALS 1.41	10.42
Crude	92310	100	0 ^(c)	N.D. ^(d)		

Recovery: untreated fractions : $\frac{1.41}{0} \times 100 = \infty$
vs. crude b.p. cut

Recovery: nitrous acid frac- : $\frac{10.42}{1.41} \times 100 = 739\%$
tions vs. untreated

- (a) Specific mutagenic activity estimated with *S. typhimurium* TA98 as target cell from a single experiment dose response experiment.
 (b) Product of wt% times specific mutagenic activity (rev/μg).
 (c) Specific mutagenic activity estimated from average of 3 experiments.
 (d) N.D. = Not determined.

TABLE 6. Mutagenicity of HPLC Fractions from B.P. 710 to 730°F Cut of Sample Set No. 1 Before and After Treatment with Nitrous Acid

Fraction	Wt (μg)	Wt%	Specific Mutagenic Activity (rev/μg) ^(a)		Weighted Specific Mutagenic Activity (rev/μg) ^(b)	
			Untreated	Nitrous Acid	Untreated	Nitrous Acid
1	60,040	76	0	0	0	0
2	140	<1	0	0	0	0
3	13,350	17	162.05	10.44	27.05	1.77
4	1,050	1	0	4.84	0	0
5	3,860	5	0	0	0	0
6	7,980	10	0	0.78	0	0.80
7	220	<1	15.94	0	0.03	0
TOTALS	86,640	109		TOTALS	27.08	2.67

Crude 79,467 100 0.53±0.42^(c) N.D.^(d)

Recovery: untreated fractions : $\frac{27.08}{0.53} \times 100 = 510.9\%$
vs. crude b.p. cut-

Recovery: nitrous acid frac : $\frac{2.67}{27.08} \times 100 = 10\%$
tions vs. untreated

(a) Specific mutagenic activity estimated with *S. typhimurium* TA98 as target cell from a single experiment dose response experiment.

(b) Product of wt% times specific mutagenic activity (rev/μg).

(c) Specific mutagenic activity estimated from average of 3 experiments.

(d) N.D. = Not determined.

TABLE 7. Mutagenicity of HPLC Fractions from B.P. 739 to 761°F Cut of Sample Set No. 1 Before and After Treatment with Nitrous Acid

Fraction	Wt (μg)	Wt%	Specific Mutagenic Activity (rev/μg) ^(a)		Weighted Specific Mutagenic Activity (rev/μg) ^(b)	
			Untreated	Nitrous Acid	Untreated	Nitrous Acid
1	62,370	69	0	0.42	0	0.29
2	320	<1	0	5.7	0	0.01
3	14,630	16	453.17	25.27	72.51	4.04
4	1,610	2	61.67	32.63	1.23	0.65
5	3,110	3	0	0	0	0
6	7,710	8	0	0.29	0	0.02
7	<u>1,190</u>	<u>1</u>	7.44	0	<u>0.07</u>	<u>0</u>
TOTALS	90,940	99			TOTALS 73.81	5.01
Crude	90,940		30.00±10.9 ^(c)	N.D. ^(d)		

Recovery: untreated fractions vs. crude b.p. cut : $\frac{73.81}{30.00} \times 100 = 246\%$

Recovery: nitrous acid fractions vs. untreated : $\frac{5.01}{73.81} \times 100 = 6.79\%$

(a) Specific mutagenic activity estimated with *S. typhimurium* TA98 as target cell from a single experiment dose response experiment.

(b) Product of wt% times specific mutagenic activity (rev/μg).

(c) Specific mutagenic activity estimated from average of 3 experiments.

(d) N.D. = Not determined.

TABLE 8. Mutagenicity of HPLC Fractions from B.P. 867 to 967°F Cut of Set No. 1 Before and After Treatment with Nitrous Acid

Fraction	Wt (μg)	Wt%	Specific Mutagenic Activity (rev/μg) ^(a)		Weighted Specific Mutagenic Activity (rev/μg) ^(b)	
			Untreated	Nitrous Acid	Untreated	Nitrous Acid
1	12,670	53	0	0.42	0	0.22
2	1,420	6	0	0	0	0
3	1,510	6	94.56	0	5.67 ^(b)	0
4	3,210	13	828.02	43.28	107.64	5.63
5	2,300	10	53.62	8.41	5.36	0.84
6	12,770	12	123.01	28.11	14.76	3.37
7	80	<1	95.98	0	0.32	0
TOTALS	23,960	88		TOTALS	133.75	10.06

26

Crude 30,769 100 98.6±22.8^(c) N.D.^(d)

Recovery: untreated fractions : $\frac{133.75}{98.6} \times 100 = 136\%$
vs. crude b.p. cut

Recovery: nitrous acid frac- : $\frac{10.06}{133.75} \times 100 = 8\%$
tions vs. untreated

(a) Specific mutagenic activity estimated with *S. typhimurium* TA98 as target cell from a single experiment dose response experiment.

(b) Product of wt% times specific mutagenic activity (rev/μg).

(c) Specific mutagenic activity estimated from average of 3 experiments.

(d) N.D. = Not determined.

FIGURE 7. Effect of Nitrous Acid on Mutagenicity of HPLC Fractions from B.P. 565° to 582°F

FIGURE 8. Effect of Nitrous Acid on Mutagenicity of HPLC Fractions from B.P. 639° to 689°F

FIGURE 9. Effect of Nitrous Acid on Mutagenicity of HPLC Fractions from B.P. 710° to 730°F

FIGURE 10. Effect of Nitrous Acid on Mutagenicity of HPLC Fractions from B.P. 739° to 761°F

FIGURE 11. Effect of Nitrous Acid on Mutagenicity of HPLC Fractions from B.P. 867° to 967°F

Three b.p. cuts from above 700°F that were mutagenically active as crude materials, (b.p. 710° to 730°F, 739° to 761°F and 867° to 967°F) all yielded mutagenically active fractions after HPLC (Tables 6, 7, 8; Figures 9, 10, 11). All three b.p. cuts showed a major peak of activity eluting in HPLC Region II. For b.p. cuts 710 to 730°F and 739 to 761°F, the elution times for these genetically active fractions were from 13 to 15 min. B.p. cut 867 to 967°F also had a major peak of activity in Region II, eluting at approximately 18 min. More than 95% of the genetic activity recovered from b.p. cuts 710 to 730°F and 739 to 761°F was in Region II with trace amounts in more polar regions. Region II from b.p. cut 867 to 967°F accounted for 82% of the activity recovered from this material with the remaining 18% found in strongly polar HPLC Region IV.

The specific mutagenic activities of fractions from the above 700°F b.p. cuts of sample set no. 1 were roughly 10- to 100-fold higher than the crude b.p. cuts, suggesting an incremental increase in concentrations of mutagens in the fractions. Recovery of mutagenic activity in HPLC fractions was greater than expected, based on mutagenic activity of the crude (unfractionated) b.p.

cuts. This contrasts with results of the reblending experiments. The contrast was least noticeable in b.p. 867 to 967°F where recovered mutagenic activity was only 1.09 times greater than expected. The ratio of observed to expected mutagenic activity increased to about 2.5 for b.p. 739 to 761°F and to about 50 for b.p. 710 to 730°F. Below 700°F, only b.p. cut 639 to 689°F showed activity and the ratio of measured/expected activity was indeterminant (Table 5, Figure 8). This is the first evidence that mutagens were present in b.p. cuts below 700°F, but failed to be expressed in Ames assay. It is noteworthy that nitrous acid did not reduce the mutagenicity of the most genetically active of the HPLC fractions from b.p. 639-689°F.

Bioassay of HPLC fractions suggests that some mutagenicity may be expected for set no. 1 materials boiling just below 700°F. If the specific mutagenic activity of the various b.p. cuts and/or fractions of the two materials were weighted and summed to estimate their maximum contribution to the genetic potency of the whole material, then b.p. cut 639° to 689°F (which constitutes about 6% by weight of total set no. 1)⁽¹⁶⁾ would contributed less than 2% to the mutagenicity of the unfractionated material. HPLC fractions from b.p. 565 to 582°F were inactive in the Ames assay (Figure 7).

Differences in recovery data between reblending and HPLC fractionation experiments require explanation. Mutagenicity in reblended (unfractionated) cuts was approximately additive. However, bioassay of HPLC fractions showed that separation of chemical classes from b.p. cuts based on polarity gave rise to fractions which were more active than could be accounted for by the mutagenicity of crudes.

Evidently, total mutagenic potency in crude b.p. cuts was not expressed, either individually or as reblended mixtures. However, degree of mutagenic inhibition (apparently as a result of chemical composition of the cuts) reached some fixed level which was approximately additive in reblended mixtures. According to this hypothesis, HPLC fractionation removed inhibiting compounds from Region II constituents, thus accounting for the increased mutagenic activity in fractions.

Both reblending and fractionation experiments have direct bearing on conclusions about how mutagenicity is distributed as a function of b.p. temperature. The reblending experiment showed that b.p. 300 to 700°F, which was itself genetically inactive and the major portion (80% by weight) of HPS, did not strongly affect the expression of mutagenicity in the remaining crude b.p. cuts of HPS. Nevertheless, masking did occur in the crude b.p. cuts. For b.p. cuts just above 700°F, the apparent inhibition of genetic activity was severe. For example, for b.p. 710° to 750°F and 730° to 761°F a high level of latent mutagenicity was expressed after fractionation. For the b.p. cuts just below 700°F, some latent mutagens were revealed but their concentrations were apparently low. The rapid increase in mutagenicity with b.p. temperature, and the apparent monotonic nature of the activity curve, suggests that concentrations of mutagens measurable in the Ames assay became undetectable at b.p. near 650°F, and were negligible up to 710 to 730°F.

Nitrous acid treatment of HPLC fractions from the b.p. cuts above 700°F reduced their mutagenicity by a factor of about 90%, a result which is diagnostic for inactivation of PAA. As shown elsewhere,^(26,43) PAA containing at least four condensed aromatic rings are present in high-boiling SRC-I and SRC-II distillates spanning the temperature range 485° to 850°F. Data presented here suggest that PAA are also determinant mutagens in boiling point cuts for temperatures above 800°F. Molecular weights corresponding to PAA of five condensed rings were major constituents of the mutagenically active HPLC fractions from b.p. cuts 730° to 761°F and 867° to 967°F, and minor constituents of less mutagenically active HPLC fractions from b.p. 710° to 730°F (see below). There was no evidence, based on mass spectrometry, for PAA of greater than three condensed rings in HPLC fractions from b.p. cut 710° to 730°F, 639° to 689°F or 565° to 585°F.

Nitrosation of b.p. 639 to 689°F (i.e., below 700°F b.p. cut) actually increased the mutagenicity of this material, suggesting that PAA were either present in such low concentrations that the direct-acting mutagens formed as a by-product of nitrosation were more active than the PAA before nitrosation; or, that a different type of mutagen (non-PAA) was present in this b.p. cut.

Region I of all HPLC chromatograms contributed the greatest fraction of mass recovered from b.p. cuts by HPLC fractionation and was also highly enriched in condensed (ring) PAH compounds. For the b.p. cuts under 700°F, the predominant PAH structures appeared to contain three or fewer aromatic rings, although smaller concentrations of pyrene (4-ring) were found. No detectable levels of 5-ring PAH were seen. Thus, if mutagens are present in this set, their mutagenicity would be expected to be weak compared to the larger (i.e., 5-ring) PAH. (14,29,34)

For the b.p. cuts above 700°F, and in particular b.p. 739° to 761°F and 867° to 967°F which showed much higher levels of mutagenic activity, PAH content increased and the average size was five or greater condensed rings. Semi-quantitatively, MS data showed that mutagenic b.p. cuts were also highly enriched in PAH compounds which individually are potent mutagens in the Ames assay.

However, the PAH fractions were totally without activity in the standard Ames assay (Tables 5 to 8 and Figures 7 to 11), suggesting that PAH compounds (e.g., BaP), although present in high concentrations in b.p. cuts are not expressed in bioassays. Evidence suggests this may be a common finding for other complex mixtures. (7,9,18,21,23,36)

CHEMICAL ANALYSIS

Chemical analyses were performed on selected boiling point cuts and their chemical subfractions resulting from the application of HPLC. These analyses were conducted in order to relate a number of chemical parameters to the observed in vitro biological test results, and to assess the effectiveness of the distillation procedure and subsequent fractionations. The main emphasis in this report is on the molecular weight distributions of polyaromatic compounds in the b.p. cuts and their HPLC fractions, and the characterization of large-ring PAA and PAH. A more complete qualitative and quantitative chemical study is underway and will be published in a subsequent status report.

In the present section we refer to the presence of molecular ions which have the proper molecular weight for partially hydrogenated PAH species. The distinction between molecular ions from partially hydrogenated PAH having a given ring structure and highly alkylated PAH having a more condensed or smaller ring system is difficult to achieve when dealing with such complex mixtures as the distillate cuts. Tetralins and partially hydrogenated phenanthrenes are known to be present in SRC coal liquids and it is reasonable to assume that larger PAH may be also partially saturated during liquefaction. However without proper standards or special separation procedures to positively identify the hydroaromatics, the question must remain open.

The probe inlet MS method used is very rapid and useful in assessing PAH ring size in the various cuts. However estimates of overall molecular weight for the fractions are likely to be low due to fragmentation and the possible failure of polar or higher molecular weight species to come off of the probe. Comparison of values obtained here with those reported in reference 5 indicate that the discrepancy averages about 15-25 amu and is greatest in the mid range b.p. cuts.

Chemical Class Fractionation

Figures 12, 13, and 14 show the weight percentages of each of the b.p. cuts from set no. 2 which partitioned into the various chemical fractions during the separation procedure shown in Figure 3. Several trends became evident from these results. First, the alkane concentration decreased with

FIGURE 12. Comparison of Aliphatic Hydrocarbon and Nitrogen PAH Content for Various Boiling Point Cuts from sample set no. 2 as Estimated by Chemical Class Fractionation. The primary aromatic amine rich subfraction of the nitrogen PAH fraction is depicted in Figure 14.

FIGURE 13. Comparison of Neutral PAH and Hydroxylated PAH Content of the B.P. Cuts from the Second Sample Set as Estimated by Chemical Class Fractionation

FIGURE 14. Increase in Relative Weight of Primary Aromatic Amine Enriched Subfraction from Nitrogen PAH Fraction in the second sample set

concomitant increases in nitrogen containing PAH concentration as the b.p. temperature of the cuts increased, implying that the higher-boiling b.p. cuts are in general more aromatic than the lower boiling ones. The relative weight of the primary aromatic amine enriched subfraction of the nitrogen fraction also increased with boiling point temperature starting with the 750° to 800°F b.p. cut.

These general characteristics in the distribution of chemical classes among the various b.p. cuts are also evident from results of standard petroleum industry methodologies as reported in reference 5.

These trends are consistent with the observed increased genotoxicity of higher-boiling fractions which were found to contain relatively large concentrations of PAH and substituted PAH (such as PAA) having 4 or more condensed rings. In a general way, these results also agree with the relationship between temperature, nitrogen content and the mutagenicity of pyrolytic products from other complex chemical mixtures. (3,11,13,17,36)

Mass Spectral Screening of Set No. 2 Crude B.P. Cuts

The five crude 50°F b.p. cuts produced from set no. 2 were characterized by probe inlet mass spectrometry to estimate the characteristic ring size and molecular weight ranges for these complex materials. (Values obtained by simply choosing the most intense peak in averaged mass spectra as the representative molecular weight; tended to be some 15 to 25 amu (ca 10Δ) below those reported in reference 5.) Figures 15 through 17 are mass spectra characteristic of the five cuts prior to HPLC fractionation. As estimated by this method the average molecular weight showed the expected increase with increasing b.p. temperature. Relative average molecular weights were assigned by comparing the mass of the base peak (most intense ion) in each of the various spectra. In b.p. cuts such as these, mass distribution about the base peak is roughly Gaussian, except for bottoms materials which were generally skewed somewhat to higher mass. Higher molecular weight compounds are discriminated against by the method due to fragmentation caused by electron impact ionization and the low volatility of higher molecular weight materials which prevents their being completely evaporated off the probe. The latter effect was most pronounced

FIGURE 15. Average Probe Inlet Mass Spectra of the 850°F⁺ (upper) and the 800° to 850°F B.P. Cuts (lower) from sample set no. 2

FIGURE 16. Average Probe Inlet Mass Spectra of the 750° to 800°F B.P. Cut (upper) and the 700° to 750°F B.P. Cut (lower) from Sample Set No. 2

FIGURE 17. Average Probe Inlet Mass Spectrum of the 300° to 700°
B.P. Cut from sample set no. 2

for higher boiling fractions. For higher boiling b.p. cuts, there appeared groups of ion peaks at one-half the m/e value from some of the prominent ions in the mass spectrum (e.g., m/e 120, m/e 123, etc.). These ions were mostly doubly charged species and are typical in the EI spectra of larger multiring and some substituted PAH. The presence of such ions indicates the highly aromatic character of these coal liquids.

Mass spectral analysis of crude b.p. cuts confirmed evidence obtained by chemical fractionation showing increased aromaticity in higher-boiling cuts, along with increased concentrations of nitrogen-containing polycyclics. Mass spectra in Figures 15 through 17 show a distinct increase in average molecular weight with each 50°F increment in b.p. temperature. The average number of aromatic rings assigned to compounds in various fractions increased from less than 3 to greater than 5 for PAH, and from less than 2 to greater than 4 for nitrogen-containing PAH, such as PAA, carbazoles and azaarenes. Large multi-ring PAH, PAA and azaarenes are logical candidates to account for the genetic activity of b.p. cuts. However, PAA appear to be the determinant microbial

mutagens. The PAH and azaarenes, although present in relatively high concentrations, are apparently minor contributors to the mutagenicity of the coal liquids.

Analysis of HPLC Fractions

Figure 18a is a high resolution capillary column GC chromatogram of the PAH fraction as obtained by HPLC from sample set no. 2 source liquid prior to distillation. The corresponding fraction from the b.p. cut is 750 to 800°F shown in Figure 18b. Comparison of the chromatograms illustrates the effect on PAH ring size distribution resulting from distillation into 50°F cuts. Table 9 lists compounds identified in the materials by GCMS.

Mass spectral analyses by GCMS, probe inlet MS or both, were performed on several fractions resulting from HPLC separation. Analyses were performed as a general survey of the kinds of compounds in the cuts, with special emphasis on the differences and definition achieved by distillation with regard to aromatic ring number. Good estimates of neutral PAH content were made for most materials based on GCMS or probe inlet MS. However, for materials which could not be analyzed by GCMS, reliable estimates of PAA content and identification of highly polar components having more than one heteroatom were difficult to obtain. However, it has been demonstrated using several SRC materials, that monofunctional PAA elute in HPLC Region II (Figure 2), along with the azaarenes and carbazoles.⁽³⁹⁾ We have also shown that for mutagenically active fractions of SRC-II heavy distillate, the PAA content of Region II (Figure 2) is about 10% of that for nitrogen heterocycles eluting in the region.

Although we intend to make detailed analysis of the active b.p. cut materials and to quantitate as far as possible their PAA and multifunctional compound content, present results include only qualitative data indicating presence of PAA. More quantitative data on PAA content from heavier non-gas chromatographable fractions will be described in a future report.

Analysis of HPLC Fractions from Sample Set No. 1 B.P. Cut 867° to 967°F

GCMS analysis was performed on HPLC fractions from b.p. cut 867° to 967°F; However, with the exception of some 5-ring compounds from fraction no. 1, no material eluted from the GC column, even at temperatures up to 340°C. GCMS

FIGURE 18. (a) Capillary Column Gas Chromatogram on HPLC Fraction No. 1 of Undistilled source material from set no. 1. Peak numbers refer to compounds listed in Table 12. Conditions: 30 m x 0.28 mm fused-silica capillary column coated with SE-54, held isothermal at 50°C for 2 min then temperature programmed from 50°C to 260°C at 2°C/min and held isothermal at 260°C for 10 min.

(b) Capillary Column Gas Chromatogram of HPLC Fraction No. 1 of sample set no. 1 with boiling point between 700°F to 750°F. Peak numbers refer to compounds listed in Table 12. Conditions: 30 m x 0.28 mm fused-silica capillary column coated with SE-54, held isothermal at 50°C for 2 min then temperature programmed from 50°C to 260°C at 2°C/min and held isothermal at 260°C for 10 min.

TABLE 9. Compounds Identified in Undistilled HPS and Distillate Cut 700°F to 750°F. Numbers refer to peaks shown in Figures 18a and 18b.

<u>Peak Number</u>	<u>Molecular Weight</u>	<u>Compound</u>
1	118	Indan
2	132	C ₁ -Indan
3	132	Tetralin (1,2,3,4-tetrahydronaphthalene)
4	128	Naphthalene
5	146	C ₁ -Tetralin
6	142	C ₁ -Naphthalene
7	160	C ₂ -Tetralin
8	154	Biphenyl
9	156	C ₂ -Naphthalene
10	170	C ₃ -Naphthalene
11	168	C ₁ -Biphenyl
12	184	C ₄ -Naphthalene
13	166	Fluorene
14	198	C ₅ -Naphthalene
15	192	C ₂ -Biphenyl
16	180	C ₁ -Fluorene
17	184	Dibenzothiophene
18	178	Phenanthrene
19	198	C ₂ -Fluorene
20	206	C ₃ -Biphenyl
21	204	Phenylnaphthalene
22	192	C ₁ -Phenanthrene
23	204	Phenylnaphthalene
24	206	C ₂ -Phenanthrene
25	202	Fluoranthene
26	202	Pyrene
27	216	C ₁ -Pyrene
28	216	Benzofluorene
29	230	C ₂ -Pyrene
30	244	C ₃ -Pyrene
31	228	Chrysene
32	258	C ₄ -Pyrene

showed the HPLC fraction 1 from the 867° to 967°F cut contained benzopyrenes and perylene as the only gas chromatographable components (Figure 19). Probe MS analysis of this fraction also indicated the presence of some chrysene or benzanthracenes, along with their C₁ and C₂ homologs, and C₁, C₂ and C₃ benzopyrenes and/or perylenes. It was possible to effect some fractional distillation of these components off the inlet probe, as indicated by two distinct envelopes in the total ion current which appeared as the temperature was increased. Spectra showing the m/e 242 (C₁ chrysene/benzanthracenes) as base peak ion in the first envelope and m/e 252 (benzopyrene/perylenes) as base peak ion in the second envelope are shown in Figure 20a and b. The second HPLC fraction from b.p. 867° to 967°F again gave a clear distillation envelope indicating at least two major classes of compounds were present. These appeared to be mainly 5 (linear)-ring species such as pentacene, dibenzphenanthrenes, etc., and condensed 6-ring anthanthrenes, etc., in the first envelope. Coronenes and dibenzofluoranthenes were the major species in the second. All of these compounds were present with alkylated homologs, and partially hydrogenated analogs also appear to be present. Figure 20c is a spectrum from the second envelope showing the m/e 316 (C₁ dibenzofluoranthene) as base peak. Coronene (m/e 300), dibenzofluoranthene (m/e 302), and C₂ dibenzofluoranthene (m/e 330) also appear to be present. Heavier compounds detected in cut no. 2 included 7 (linear)-ring compound at m/e 378 and ovaline at m/e 398. The m/e 378 ion was at 10% base peak height in several spectra from the second envelope.

HPLC fraction no. 3 (Region II) from the 867° to 967°F cut contained mostly larger-ringed aza compounds including azabenzpyrenes/azaperylenes and their C₁ homologs (m/e 267) and C₂ (m/e 281) and a series of ions at m/e 283, 297, 311, etc. which were identified as alkylated azacyclopentaceanthrylenes and/or alkylated dihydro azabenzpyrenes. Ions having the proper mass-to-charge ratio to represent the corresponding dihydro/tetrahydro series were also present (e.g., m/e 285, 299, 313, 327, etc., Figure 21). HPLC fraction no. 4 (also Region II) did not appear to be substantially different in composition from no. 3 with respect to aza compounds present. Ions such as m/e 267, m/e 281, m/e 295, etc., also arise from amino benzpyrenes. Analysis

FIGURE 19. Reconstructed Total Ion Chromatograms from HPLC Fraction No. 1 of a) b.p. 867° to 967°F, b) b.p. 710° to 730°F and c) b.p. 639° to 689°F with Major Constituents Identified.

FIGURE 20a. Spectra Obtained from HPLC Fraction No. 1 of the 867° to 967°F b.p. cut Illustrating Kinds of Compounds Not Amenable to GC but Which Distilled Off the MS Heated Probe Inlet. Peaks in (a) are primarily benzantracenes, chrysenes, and their alkyl homologs; peaks in (b) are mostly benzopyrenes, pyrenes, and their alkyl homologs; peaks in (c) are coronenes and benzopyrenes together with their alkyl homologs. Peaks corresponding to molecular weights for partially hydrogenated species are evident in the latter two spectra.

FIGURE 20b,c.

FIGURE 21. Average Probe Inlet Mass Spectrum of HPLC Fraction No. 3 in the Moderately Polar Region from B.P. 867 to 967°F. Ion peaks in this region may represent one or more of several structures. The m/e 267, for example, has been shown to have contributions from aminobenzopyrenes, dibenzcarbazoles, and methyl azabenzopyrenes. The m/e 281 may contain alkyl homologs of all of these components.

of SRC-II heavy distillate by metastable ion energy spectrometry has demonstrated the presence of this compound,⁽⁴⁵⁾ and it can be assumed to be a component of HPLC fraction no. 3 for b.p. 867° to 967°F.

B.p. 867° to 967°F was not amenable to GCMS. Thus GC retention time could not be used to distinguish the polycyclic PAA in these fractions from the substantially more abundant aza compounds and carbazole benzologs. Most primary amines have elemental composition identical to their corresponding alkylated nitrogen heterocycle and yield the same molecular ions in mixtures such as those studied here. To obtain a rough estimate of the relative PAA content,

HPLC fractions no. 3 and no. 4 from b.p. 867° to 967°F were derivitized with a silanization reagent. Removal of molecular ion peaks by this method was taken to indicate presence of PAA. The procedure left exclusive azaarene ions such as m/e 203 and m/e 253 at relatively higher intensities indicating that ions such as m/e 217, 231, 267, etc., were due in part to presence of PAA since their relative intensity had been diminished by derivitization.

HPLC fraction no. 5 from the 867° to 967°F cut contained a relatively large number of compounds. However, because it could not be analyzed by GCMS, identification of many of the ions is not certain. Compounds for which structural assignment was straightforward included azabenzopyrene (a major constituent in fraction no. 4), hydroxybenzpyrene, C₁ hydroxybenzpyrene, hydroxy-azabenzanthracene/hydroxyazachrysene and their C₁ and C₂ homologs and possible dihydro and tetrahydro analogs for each of these compounds. Tentative identifications include hydroxyazabenzopyrene and a homologous series of hydro-pyrenes from C₂ to C₅, and a series of partially hydrogenated hydroxy benzanthracenes and/or benzofluoranthenes from C₁ to C₆. Many of these compounds also appeared in fraction no. 6 along with apparent dihydroxy compounds as well as species which were tentatively identified as hydroxylated PAA.

Mass Spectral Analysis of Set No. 1 B.P. Cut 739° to 761°F

HPLC fraction no. 1 of set no. 1 b.p. cut 739° to 761°F was comprised mainly of pyrene and alkylated pyrenes, the m/e 216 peak from C₁ pyrene or benzanthracene/benzofluorene being the base peak for the spectrum. Compared with b.p. 710° to 730°F, there appeared to be fewer partially hydrogenated species. Fraction no. 2 contained little material and was comprised mostly of partially hydrogenated C₁ and C₂ pyrenes and benzanthracenes. An anomaly in the composition of fraction no. 2 was the presence of a benzovalene or its isomers. The molecular weight of this compound was 448 and it yielded an excellent mass spectrum with the requisite doubly charged m/e 224 co-evaporating off the probe. The reason for the presence of this compound in b.p. 739° to 761°F is not clear.

HPLC fraction no. 3 contained azapyrenes and/or aminopyrenes and C₁ and C₂ homologs. Although b.p. temperature ranges for the 710° to 730°F and the 739° to 761°F cuts were similar, there appeared to be more heterogeneity in HPLC fraction no. 3 of the latter. The phenanthroline/phenazine series at m/e 180 and m/e 194 were present. HPLC fraction no. 4 was not substantially different from no. 3. Fraction no. 5 contained little material; however, spectra were present from both hydroxylated compounds and compounds containing two nitrogen atoms. Fractions no. 8 and no. 9 contained almost exclusively hydroxylated compounds, the principal ones being alkylated hydroxy biphenyls. Alkylated dihydroxy biphenyls were principal components of fraction no. 10.

GCMS Analysis of HPLC Fractions from Sample Set No. 1 B.P. Cut 710° to 730°F

HPLC fraction no. 1 from b.p. 710° to 730°F contained almost exclusively pyrene (Figure 18), C₁ and C₂ pyrenes together with cyclopenta phenanthrenes and/or partially hydrogenated analogs of these compounds. Fraction no. 2 contained little material although some alkyl pyrenes were detected by GCMS.

Derivatization was performed on HPLC fraction no. 3 to estimate PAA content. Products of derivatization had enhanced GC characteristics compared to the non-derivatized materials. $[\text{CH}_2\text{N}(\text{SiMe}_3)_2]^+$, characteristic of silanized primary amines, was monitored to check for PAA. PAA identified in the 710 to 730°F HPLC fraction no. 3 were mainly 3-ringed compounds such as aminophenanthrene and aminofluorene as identified by GC retention time.

A series of compounds having two nitrogen atoms according to HRMS measurements, tentatively identified as either phenazines or phenanthrolines (m/e 180) were also detected in the 710° to 730°F cut of HPLC fraction no. 3. Benzoquinolines were considered as possible structures for this compound. However, the spectra did not exhibit the required M-28 fragment ion (loss of N₂). The compound(s) appeared to be present with both their alkyl and primary amino analogs, m/e 194 and m/e 223, respectively. The latter compound could be silanized to give a strong m/e 295 ion which yielded the expected m/e 222 fragment ion (loss of one TMS group). We were unable to determine whether mutagenicity of any of these proposed structures had been studied. As in the 867° to 967°F b.p. cut, the HPLC no. 4 fraction did not substantially differ

in general composition from no. 3. HPLC fraction no. 5 of the 710° to 730°F cut yielded a group of GC peaks eluting over a narrow temperature range which could not be resolved on a 30-m fused silica column. Severe tailing which characterized several of these peaks suggested they were comprised partially of compounds with polar substituents such as hydroxyl or primary amino functionalities. Spectra obtained from the GC traces were clean and matched those of C₁ and C₂ amino carbazoles. Diaminophenazine was also considered a possible structure for one compound which had an intense m/e 210 ion. However, the spectra obtained did not match published standard spectra as did the spectra of amino carbazoles. The fact that these GC peaks could not be resolved and that several of them yielded essentially identical mass spectra, supports the view that these compounds may be alkylated amino carbazoles. The large number of positional isomers arising from the presence of two or three substituent groups would not be expected to separate chromatographically on the column employed.

Mass Spectral Analysis for Set No. 1 B.P. Cut 639° to 689°F

Phenanthrenes, anthracenes, and their C₁ and C₂ homologs were primary constituents of HPLC fraction no. 1 from the 639° to 689°F b.p. cut. Fraction no. 2 of this material contained alkylated 3-ring PAH and some pyrene. However, there was relatively little material in this HPLC fraction. HPLC fraction no. 3 was comprised mainly of carbazoles and alkylated carbazoles with some phenanthridine and/or acridine. Fraction no. 4 was not substantially different from no. 3 except for the appearance of compounds containing two nitrogen atoms such as phenanthroline. Fraction no. 5 contained some carbazoles, phenanthrolines and hydroxylated 3-ringed components at m/e 194. Note that for b.p. 639° to 689°F, the fraction designation is altered with respect to other b.p. cuts and that fraction no. 3 is in a transition region between moderate and high polarity.

In summary, MS analysis of HPLC fractions of b.p. cuts showed a strong correlation between nitrogen-containing polycyclics in a fraction and mutagenicity of that fraction. Moreover, as genetic activity of the fractions increased, average ring size also increased from approximately 2 (least active) to 5 (most genetically active). Nitrosation data suggest the active nitrogen-

containing species in these fractions were PAA. This could not be confirmed by direct probe MS analysis. However, derivitization studies, coupled with GCMS analysis, confirmed the identity of large-ring PAA in some genetically active HPLC fractions and will be the subject of a future report.

The converse situation appeared to be true for PAH. High concentrations of PAH of 5 or more rings appeared to be present in higher-boiling cuts of HPLC fractions. In particular, BaP appeared to be a major constituent of these materials and there was genetic activity in the HPLC fractions. Thus, mutagenicity of the large-ring PAH was severely reduced in the mixtures (i.e., chemical environments) represented by HPLC fractions.

Results of HRMS Analysis

HRMS analyses have not been completed for all of the fractions discussed above. In general, however, HRMS shows that for similar crude materials such as SRC-II heavy distillate and SRC-I process solvent, heteroatom content increases with polarity, and with boiling point. Results of HRMS analyses on these samples will be included in a future report. Application of HRMS to SRC-II liquids other than heavy distillate has been limited to confirming elemental composition of principal compounds containing one or two nitrogen atoms and a survey of heteroatom content. For example, compounds containing N, O, S, N+O, N+S, S+O, and N+O+S have been identified. Exclusive N-containing compounds are generally confined to the moderately polar regions such as HPLC fractions nos. 3 and 4. Some exclusive S-containing compounds and furans elute in fraction no. 2. The remaining species elute in the highly polar regions of HPLC chromatograms.

Results of Derivitization for Specific Detection of PAA

The 300° to 700°F blend from sample set no. 2 was fractionated by chemical class and the S-2 fraction containing PAA was derivitized as described in reference 47. Analysis of the sample by GC/ECD showed the presence of PAA. Major components were identified by their relative GC retention times and molecular weights from GCMS analysis as aminoindans, aminonaphthalenes, and aminobiphenyls. Major gas chromatographable components in the corresponding 700°

to 850°F blend were shown to be aminofluorenes, together with their C₁ and C₂ homologs, aminophenanthrenes/aminoanthracenes and their C₁ homologs, and aminopyrenes. Results from the GCMS analysis for the 700°–850°F blend are shown in Figures 22 and 23, which illustrate reconstructed ion chromatograms for the molecular ions from the PFP derivatives of the major components.

RELATIVE ION INTENSITY

FIGURE 22. Selected Ion Chromatograms for the m/e 327, m/e 341 and m/e 355 Molecular Ions From the PFP Derivatives of Aminofluorene and its C_1 and C_2 Homologs Respectively, From the 700° to 850°F Blend of Sample Set No. 2.

FIGURE 23. Selected Ion Chromatograms for the m/e 339, m/e 353, and m/e 363 Molecular Ions From the PFP Derivatives of Aminophenanthrenes/ Aminoanthracenes and their C₁ Homologs, and Amino Pyrene Respectively, From the 700° to 850°F Blend of Sample Set No. 2.

CONCLUSIONS

The crude cuts from both sample sets boiling under 700°F showed no genetic activity in the microbial assays used. About 80% by weight of the material in set no. 2 boiled under 700°F. Comparable data on weight distribution were not available for sample set no. 2. However, agreement between mutagenicity and b.p. cut temperature range for the two materials suggests that at least 80% by weight was either non-mutagenic or weakly mutagenic in the Ames or prophage induction assays. Thus, most of the SRC-II material produced under conditions designed to simulate demonstration facility operation may be recovered as a liquid product of low mutagenic potency providing higher-boiling constituents are removed.

The genetically inactive b.p. cuts boiling under 700°F did not appear to exert strong effects on the mutagenic potency of the cuts boiling above 700°F b.p. cuts, although slight synergism was suggested from the reblending experiments. However, the genetic activity observed for reblended cuts closely approximated their predicted activity based on the calculated additivity of the individual b.p. cuts.

Conversely, additivity of genetic effects was not observed for the HPLC fractions derived from individual b.p. cuts. The fractions derived from the most mutagenic of the b.p. cuts accounted for more activity than expected from the amount of crude fractionated. Evidently the full potential for mutagenicity was not expressed in the crude b.p. cuts. The greatest masking of genetic activity appeared to be localized in b.p. cuts representing the temperature region between 700° and 800°F. This temperature range represents the transition from b.p. cuts of low to high mutagenicity. For the b.p. cuts above 800°F the activity of the HPLC fractions was nearly additive and yielded approximately the value expected for the crude b.p. cut.

Apparent masking of genetic activity was also detected for compounds from set no. 1 boiling just below 700°F; i.e., materials that did not exhibit mutagenicity as crude b.p. cuts. However, the total contribution of this material to mutagenicity of unfractionated source material was small, estimated at less than 2% of the total. No mutagenic activity was detected in b.p. cuts below 600°F.

Thus, the bioassay data obtained from the crude b.p. cuts and from the HPLC fractions of selected b.p. cuts are generally in accord, in that very little genetically active material was produced below a temperature of 700°F. Between 740° and 760°F the mutagenicity of the materials began to increase rapidly. Masking of activity did occur, and was most severe in the latter temperature interval.

Nitrous acid destroyed more than 90% of the mutagenicity associated with HPLC fractions derived from the above 700°F b.p. cuts. As shown elsewhere, nitrous acid inactivation of mutagenicity in SRC-II liquids is diagnostic for condensed ring PAA.^(25,48) The nitrous acid data presented here agree with these observations. In addition, mass spectral analysis of the inactive, weakly active and strongly active b.p. cuts showed a positive relationship correlating PAA content, number of aromatic rings and genetic activity. For example, one of the most active b.p. cuts appeared to contain the highest concentration of 4- and 5-ring PAA, and the highest concentration of total PAA.

As expected, higher molecular weight compounds (including PAA) appear to be concentrated in the above 700°F b.p. cuts. This was particularly evident for the large condensed ring compounds which were found exclusively in the higher-boiling b.p. cuts. Mass spectral analysis showed that a significant proportion of these PAH were either benzo[a]pyrene (BaP) or isomers of BAP. However, although the determinant Ames-test genotoxins (PAA) were expressed when contained in a given b.p. cut, large-ring PAH that would have been mutagenic as pure compounds in solution were not expressed in the chemical environment represented by HPLC fractions resulting from such cuts.

These findings and conclusions, particularly if confirmed by in vivo skin painting studies, may provide insight into the chemical species that actually determine the genetic toxicity of the SRC-II high-boiling liquids. They may also aid in determining optimum operating parameters for product distillation in the proposed SRC-II demonstration facility. Assessment of the economic and technical feasibility of this approach will require input from engineers and plant operators.

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