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**Fractionally Distilled SRC-I,
SRC-II, EDS, H-Coal and ITSL
Direct Coal Liquefaction Process
Materials: A Comparative
Summary of Chemical Analysis
and Biological Testing**

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July 1985

**Prepared for the U.S. Department of Energy
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**Pacific Northwest Laboratory
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FRACTIONALLY DISTILLED SRC-I, SRC-II, EDS,
H-COAL AND ITSL DIRECT COAL LIQUEFACTION
PROCESS MATERIALS: A COMPARATIVE SUMMARY
OF CHEMICAL ANALYSIS AND BIOLOGICAL TESTING

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FOREWORD

This document reports and compares the results compiled from chemical analyses and biological testing of coal liquefaction process materials which were fractionally distilled, after production, into various comparable boiling-point range cuts. Comparative analyses were performed on solvent refined coal (SRC)-I, SRC-II, H-Coal, EDS and integrated two-stage liquefaction (ITSL) distillate materials. Mutagenicity and carcinogenicity assays were conducted in conjunction with chromatographic and mass spectrometric analyses to provide detailed, comparative, chemical and biological assessments. Where possible, results obtained from the distillate cuts are compared to those from coal liquefaction materials with limited boiling ranges. Work reported here was conducted by investigators in the Biology and Chemistry Department at the Pacific Northwest Laboratory (PNL), Richland, WA.

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LIST OF ABBREVIATIONS

A ₁	= Aliphatic hydrocarbon alumina column fraction
A ₂	= Neutral PAH alumina column fraction
A ₃	= NPAC alumina column fraction
A ₄	= Hydroxy-PAH alumina column fraction
AH	= Aliphatic hydrocarbon(s)
amino-PAH	= Amino-substituted polycyclic aromatic hydrocarbon(s)
amu	= Atomic mass unit
APHA	= American Public Health Association
API	= American Petroleum Institute
BaP	= Benzo[a]pyrene
bp	= Boiling point
CHO	= Chinese hamster ovary
cm	= Centimeter(s)
°C	= Degrees Celsius
°F	= Degrees Fahrenheit
DMSO	= Dimethylsulfoxide
DOE	= U.S. Department of Energy
ECD	= Electron capture detector
ECLP	= EDS Coal Liquefaction Pilot Plant
EPA	= Environmental Protection Agency
FID	= Flame ionization detector
g	= Gram(s)
GC	= Gas chromatography
GC/MS	= Gas chromatography/mass spectrometry
HD	= Heavy distillate
HP	= Hewlett-Packard
HPLC	= High-performance liquid chromatography
HRGC	= High-resolution gas chromatography
hydroxy-PAH	= Hydroxy-substituted polycyclic aromatic hydrocarbon(s)
IBP	= Initial boiling point
ID	= Inside diameter
I/P	= Initiation/promotion
ISTD	= Internal standard
ITSL	= Integrated two-stage liquefaction
kg	= Kilogram(s)
L	= Liter(s)
LC ₅₀	= Median lethal concentration
LVMS	= Low-voltage probe-inlet mass spectrometry

m	= Meter(s)
mg	= Milligram(s)
µg	= Microgram(s)
ml	= Milliliter(s)
µl	= Microliter(s)
mm	= Millimeter(s)
µm	= Micrometer(s)
MS	= Mass spectrometry
m/z	= Mass-to-charge ratio
ng	= Nanogram(s)
NIH	= National Institutes of Health
nm	= Nanometers
NPAC	= Nitrogen-containing polycyclic aromatic compound(s)
NPD	= Nitrogen-phosphorus detector
PAH	= Polycyclic aromatic hydrocarbon(s)
PAC	= Polycyclic aromatic compound(s)
PDU	= Process development unit
PFPA	= Pentafluoropropionic anhydride
PMA	= Phorbol myristate acetate
PNL	= Pacific Northwest Laboratory
ppm	= Parts per million
ppt	= Parts per thousand
PS	= Process solvent
psig	= Pounds per square inch gage
rpm	= Revolutions per minute
S9	= Metabolic enzyme system used in microbial mutagenicity assay
SD	= Standard deviation
sec	= Second(s)
SRC	= Solvent refined coal
TA98	= Designation for strain of <u>Salmonella typhimurium</u> used in microbial mutagenicity assay
TC	= Total carbon
TLP	= Total liquid product
UV	= Ultraviolet
VGO	= Vacuum gas oil
WSF	= Water-soluble fraction(s)
Wt%	= Weight percent
2-AA	= 2-Aminoanthracene

EXECUTIVE SUMMARY

Data on the chemical composition and toxicologic activity of narrow boiling point (bp) range distillate cuts from fuels produced by the solvent refined coal (SRC)-I and -II processes, as well as by the H-Coal, EDS and integrated two-stage liquefaction (ITSL) processes, have been compiled from previously published Pacific Northwest Laboratory (PNL) Status Reports and peer-reviewed literature.

The coal liquefaction materials studied represented several process types. The SRC processes were both noncatalytic. The H-Coal process utilized a catalyst in the thermal reactor. In early configurations, a catalytic hydrotreater was used to improve recycle solvent quality in the EDS process. The plant was also operated so that the entire distillate product was hydro-treated. The ITSL is a two-stage process comprising a thermal reactor and a hydrotreater. The purpose of this report is to compare the fractional distillate cuts from the processes described above.

Biological analyses of crude, 50°F bp distillate cuts of coal liquefaction materials from the SRC-I, -II, H-Coal, EDS and ITSL processes showed the general trend of increasing activity with increasing bp temperature. Elemental analyses showed that, as bp increased, the hydrogen:carbon ratio of the coal liquids decreased, with a corresponding increase in heteroatomic (nitrogen, oxygen and sulfur) species. In terms of the chemical class fractions isolated from the distillate materials, aliphatic hydrocarbon (AH) content decreased, polycyclic aromatic hydrocarbon (PAH) content remained approximately constant, and nitrogen-containing polycyclic aromatic compounds (NPAC) and hydroxy-PAH content increased with increasing bp temperature. Higher levels of neutral PAH fraction content (which includes both hydrogenated and alkylated PAH species) were present in the coal liquids from catalytically hydrogenated processes, i.e., greater than 50% for the ITSL, EDS and H-Coal materials versus less than 50% for the SRC materials.

Regardless of the process technology, the microbial mutagenicity of the 50°F distillate cuts generally increased with increasing temperature. The

microbial mutagenicity, associated mainly with the NPAC fractions of the coal liquids, tended to correspond with the detection of three- to four-ringed amino-PAH. Amino-PAH content and, hence, microbial mutagenicity, was significantly less for the EDS and ITSL coal liquefaction materials as compared to the SRC and H-Coal materials. Neutral PAH that gave rise to microbial mutagenicity were isolated in the high-performance liquid chromatographic subfractionation of the PAH fraction; the most active subfractions contained the highest concentration levels of benzo[a]pyrene (BaP).

Significant tumor-initiating activity was found for all coal liquids boiling above 700°F. As bp increased, both the tumor incidence and total number of tumors increased; the highest boiling distillate was the most potent skin tumor initiator for all the materials. The neutral PAH fractions of both the SRC-II 800-850°F and 850°F+ distillates showed the greatest amount of initiating activity as compared to their respective AH, NPAC and hydroxy-PAH chemical class fractions. In addition, the high-performance liquid chromatography (HPLC) subfractions of the active PAH fractions that contained the highest levels of BaP were the most active in the initiation/promotion (I/P) assay for mouse skin tumorigenicity.

The highest bp distillates of the SRC-I, -II and ITSL materials gave the highest mutagenic response in the Chinese hamster ovary (CHO) cell assay. Similarly to the I/P results, the PAH fractions were the most active chemical class fraction in this biological assay.

Even though there were fewer water-soluble constituents present in the higher boiling materials, those present appeared to exhibit greater toxicity to Daphnia magna than the water-soluble constituents derived from the lower boiling range cuts.

The distillates boiling above 700°F were mainly composed of four- to six- (or more) ringed polycyclic aromatic compounds (PAC) plus their alkylated homologs. Characterization of the SRC and H-Coal chemical class fractions demonstrated that similar species were present in these coal liquefaction process materials. EDS and ITSL materials tended to contain chemical species that were more highly alkylated and hydrogenated and contained lower concen-

trations of amino-PAH and azaarene content compared to process materials without catalytic hydrogenation. In addition, the EDS materials had a slightly higher average molecular weight than corresponding materials of the same distillation range from other processes. Phenyl-substituted PAC (such as phenyl-naphthalene and hydroxybiphenyl) were found in significantly less concentration in the EDS materials as compared to the SRC-II or H-Coal materials.

INTRODUCTION

BACKGROUND

The U.S. Department of Energy (DOE) has supported research and development of direct coal liquefaction technology to produce petroleum-substitute fuels from abundant domestic supplies of coal. As a component of this overall program, beginning in the 1970s, DOE-funded process developers, in conjunction with Pacific Northwest Laboratory (PNL) and other national laboratories, have cooperated in efforts to define potential health effects and industrial hygiene concerns associated with coal liquefaction technology. It has been demonstrated that, overall, coal-derived products exhibit greater mutagenic and carcinogenic potential in laboratory assays than do analogous petroleum-based materials (PNL, 1979; Kimball and Munro, 1981; Guerin et al., 1981; Calkins et al., 1983; Mahlum et al., 1983; Later et al., 1983b). In the process of defining specific toxicologic effects of coal-derived materials and identifying those compounds that give rise to such effects, process strategies became apparent for reducing or eliminating toxicologic activity (Later et al., 1983b).

From research supported by DOE, major classes of compounds have been identified that are responsible for mutagenic and carcinogenic effects in laboratory assay systems. For example, the amino-substituted polycyclic aromatic hydrocarbons (amino-PAH) have been found to be the most active chemical class using the microbial mutagenicity screening assay (Wilson et al., 1980; Guerin et al., 1980; Later et al., 1983a). Polycyclic aromatic hydrocarbons (PAH) with four or more rings have been identified as the most active chemical class in coal-derived materials that gives rise to skin tumors in laboratory animal tests (Later et al., 1983a; Mahlum et al., 1984). The nitrogen-containing polycyclic aromatic compounds (NPAC) have also been shown to be tumorigenic in both the initiation/promotion (I/P; Mahlum et al., 1983, 1984) and chronic (Renne et al., in press) assays but are less potent than their nonfunctional PAH counterparts. The observation that the biologically active constituents of these chemical classes may be concentrated in products with boiling-point (bp) ranges above about 700°F has been one of the key findings of DOE-funded health effects research (Pelroy and Wilson, 1981; Wilson et al., 1982b; Mah-

lum, 1983a,b; Mahlum et al., 1983; Renne et al., in press). Hence, distillation of full-boiling-range coal-derived products into 50°F bp cuts for combined biology/chemistry investigations at PNL has become a protocol task in the toxicologic evaluation of coal liquefaction materials from different processes. The 700°F temperature will be used in this report as the demarcation temperature between "active" and "nonactive" distillate cuts; however, this may vary by about $\pm 50^\circ\text{F}$, depending on the sample and on the definition of "toxic" in the tests described. For a detailed discussion, see Fillo and Craun (1985).

Coal liquefaction process materials contain a myriad of individual chemical components. Generally, some form of preseparation is necessary to obtain reliable chemical analyses and biological test data. Fractional distillation into discrete fractions of limited bp range is one means of reducing the number of chemical constituents present in analytes. Following initial studies with fractionally distilled solvent refined coal (SRC)-II bp cuts (Pelroy and Wilson, 1981), full-boiling-range liquefaction products from other processes were distilled into 50°F fractions for similar chemical and biological studies. Subsequent investigations have been conducted using distillation cuts with similar bp ranges from the SRC-I (Wilson et al, 1982a), EDS (Later et al., 1984), H-Coal (Wilson et al., 1985), and Lummus integrated two-stage liquefaction (ITSL; Wilson et al., 1983a) processes. In general, the results of these studies support the initial findings with the SRC-II distillate cuts: that biological activity in those laboratory screening assays used at PNL is confined to distillate fractions boiling above about 700°F. Calkins et al. (1983) have obtained analogous toxicologic results on process-distilled, coal-derived products from SRC-II, nonhydrotreated EDS and H-Coal processes. (Process distillation represents a crude distillation performed at the coal liquefaction plants; these distillation results are not expected to be as discrete as for the postproduction laboratory distillations performed on the samples described in this report.) In addition, data obtained using one set of redistilled petroleum- and three sets of shale-derived materials indicated increased biological activity in higher boiling materials.

CURRENT WORK

The purpose of this document is to present and compare the results of the chemical and biological investigations performed with the four sets of 50°F distillate cuts from the SRC-I, -II, EDS and H-Coal processes. Results from selected broader-bp-range process distillates from the Lummus ITSL process are also included. Since detailed process, chemical, and biological data are available in previous PNL Status Reports (Pelroy and Wilson, 1981; Wilson 1982a; Later et al., 1984; Wilson et al., 1985; Wilson et al., 1983a) for each set of distillate fractions, emphasis here will be placed on comparing the chemical and biological results. Furthermore, new biological data from the I/P tumorigenicity assay for the H-Coal distillate cuts, as well as the results of comparative ecotoxicity studies with reblended bp cuts from the SRC-II, EDS and H-Coal processes, will be presented and discussed in this Status Report. A brief description of each process and the source of the sample sets is also included in this document for reference purposes. The gross chemical properties, the chemical class distribution, and the qualitative/quantitative data for individual constituents in the chemical classes will also be compared. Finally, biological data for each set of distillate cuts from the microbial mutagenicity, I/P and aquatic toxicity assays will be presented, compared and discussed.

ENGINEERING

A brief description of each coal liquefaction process from which distillate cuts were obtained is given in this section as background information. More detailed process configuration and operation parameters are provided in the respective PNL Status Reports: SRC-I (Wilson et al., 1982a); SRC-II (Pelroy and Wilson, 1981); EDS (Later et al., 1984); H-Coal (Wilson et al., 1985); and ITSL, Lummus (Wilson et al., 1983a). The compilation by Baughman (1978) on different coal liquefaction processes is also an excellent source for detailed process information.

SRC PROCESSES

The development of the SRC processes was conducted on a pilot plant scale by Catalytic, Inc. and Southern Company Services (SRC-I), Wilsonville, AL; Pittsburg & Midway Coal Mining Co. (SRC-I and -II), Ft. Lewis, WA; and Gulf Science and Technology Co. (SRC-II), Harmarville, PA. All of these coal liquefaction projects received funding support from DOE. SRC-I and -II distillate materials considered in these studies were from the Harmarville and Wilsonville pilot plants, respectively.

Both SRC processes were single-stage, noncatalytic technologies. The SRC-I process (less severe than SRC-II) converted high-sulfur, high-ash coals to low-sulfur, low-ash solid fuels with minor amounts of liquid products. The SRC-II process produced a liquid rather than a solid product. The SRC-II process was less complex than the original SRC-I process and eliminated the filtration steps of the earlier technology. Schematic diagrams of the SRC-I and -II processes are shown in Figures 1 and 2 (Baughman, 1978), respectively. Representative full-boiling-range materials from the SRC-I and -II processes were fractionally distilled, chemically analyzed, and underwent toxicologic testing for these studies.

FIGURE 1. Flow Schematic of the SRC-I Process (Baughman, 1978)

FIGURE 2. Schematic Diagram of the SRC-II Process (Baughman, 1978)

EDS PROCESS

The EDS Coal Liquefaction Pilot Plant (ECLP), operated by Exxon Research and Engineering Co. at Baytown, TX, was designed to process 200 tons/day of dry coal. The plant has been operated with lignite, sub-bituminous and bituminous coals to produce a full range of products and by-products. A simplified flow schematic of the EDS process is shown in Figure 3. The EDS process is distinguished from earlier single-stage, direct coal liquefaction processes by the use of a hydrotreater to produce distilled, catalytically hydrogenated recycle solvent (hydrogen-donor solvent). The EDS design (see Figure 3) allows for a high degree of flexibility in feed coal, process configuration and product slate selection.

The EDS process has been successfully operated in two basic modes. The first distillate recycle mode was the "once-through" or "coal only" mode. In this mode, the process solvent (PS) was distilled, hydrogenated, light vacuum gas oil (VGO), which was recycled to the front-end of the process for coal slurry. In the second, or "bottoms recycle" mode, vacuum bottoms were mixed with the hydrogenated PS and feed coal in the slurry drier prior to entering the liquefaction reactor. Furthermore, in this mode, heavy VGO was added to the light VGO prior to hydrogenation and distillation for production of the recycle PS. A set of 50°F distillate cuts obtained from distillation of a blend of materials produced during operations in the bottoms recycle mode was analyzed for this work.

H-COAL PROCESS

The H-Coal direct liquefaction process was a single-stage technology developed by Hydrocarbon Research, Inc. and Cities Service Oil Co. It was based largely on H-Oil technology similar to that used to convert heavy petroleum resids to lighter products. In contrast to single-stage processes such as SRC-I and -II, which used no added catalyst in the liquefaction reactor, the H-Coal process employed a catalyst that was removed and replenished in a batch mode. The process was developed initially between 1964 and 1973, when plans were drafted to use bench-scale and process development unit (PDU)-derived

FIGURE 3. Flow Schematic for the EDS Process

processing data to build a 600-ton/day pilot plant at Catlettsburg, KY. The pilot plant was operated between 1980 and 1982.

Figure 4 is a simplified schematic of the H-Coal process (Baughman, 1978). Crushed dried coal was slurried with a recycle solvent and hydrogen was added at approximately 200 atmospheres. Slurried material was fed continuously into the ebullated-bed liquefaction reactor. Portions of the reactor catalyst were periodically removed and replaced with fresh catalyst in a batch mode. At the pilot plant, catalyst was replaced at a rate between 1 and 2 lb/ton of feed coal. The H-Coal pilot plant was operated only in the syncrude mode. In this mode, the space velocity in the reactor was nominally 30 lb of coal/hour/cubic foot of reactor volume. Nominal reactor temperature was 850°F, with the reactor operated at a pressure of approximately 3000 psig. A blend of light and heavy product oils from this process were fractionally distilled for these studies.

LUMMUS ITSL PROCESS

ITSL processes rely on a low-severity, short-contact-time initial liquefaction step, followed by antisolvent or critical solvent deashing and catalytic hydrogenation in the final liquefaction step. Resids and underflow from the hydrogenation and/or deashing operations, respectively, make up the recycle solvent used to slurry fresh coal entering the process. Product materials studied here were from the C. E. Lummus plant in New Brunswick, NJ.

A schematic flow diagram of the Lummus ITSL process is shown in Figure 5. The Lummus PDU was capable of converting 135 to 320 kg of dry coal per day. It consisted of three sections: coal extraction, antisolvent deashing of coal extract, and catalytic hydrocracking. More detail on the exact operating parameters of these three units was reported by Wilson et al. (1983a). Process streams identified by circled numbers in Figure 5 were selected for continuous sampling to closely monitor process changes from start-up to steady-state operation. Selected broad-range process distillation cuts of the total liquid product (TLP) stream (#4) were analyzed for these studies. (The process distillation of the ITSL materials represents a crude distillation performed as the products were produced at the pilot plant; these distillation results are

FIGURE 4. Schematic of the H-Coal Process (Baughman, 1978)

not expected to be as discrete as the postproduction laboratory distillations performed on the SRC-I, -II, EDS and H-Coal materials.)

FIGURE 5. Schematic Flow Diagram of the ITSL Unit

SAMPLE MATERIALS

In general, 50°F bp cuts were distilled from full-boiling-range materials that were representative of their respective liquefaction process technologies. Table 1 provides a listing of those distillate cuts studied and discussed in this report. These samples were all acquired from pilot plant or PDU test facilities and should not necessarily be considered representative of coal liquefaction materials that may eventually be produced at commercial-scale facilities. Additional information on coal type and distillation conditions is provided in the remainder of this section for each set of distillate cuts.

TABLE 1. Distillate Cuts from the Various Direct Coal Liquefaction Processes Used in Combined Biology/Chemistry Studies

Process	Distillate Cuts (°F)				
SRC-I (PNL Sample No.)	650-700 (3399-048)	700-750 (3399-049)	750-800 (3399-050)	800+ (3399-051)	
SRC-II (PNL Sample No.)	300-700 (3399-026)	700-750 (3399-027)	750-800 (3399-028)	800-850 (3399-029)	850+ (3399-030)
EDS (PNL Sample No.)	650-700 (5226-037)	700-750 (5226-038)	750-800 (5226-039)	800+ (5226-040)	
H-Coal (PNL Sample No.)	650-700 (5226-094)	700-750 (5226-110)	750-800 (5226-111)	800-850 (5226-112)	850+ (5226-109)
ITSL (PNL Sample No.)	500-650 (3399-120)		650-850 (3399-121)		850+ (3399-122)

SRC-I

The PS was the full-boiling-range SRC-I product used for distillation into 50°F bp cuts. The SRC-I PS used in these studies was produced at the

Wilsonville, AL pilot plant, during operation with Kentucky No. 9 coal from the Fies mine. The PS had a nominal bp range of 450-850°F and contained a small amount of 850°F+ heavy ends. The material was distilled into 50°F bp cuts by Air Products, Inc. research facility in Linwood, PA.

SRC-II

The SRC-II sample set consisted of distillate cuts ranging in temperature from 300°F to the 850°F+ bottoms. The distillation feed material was obtained from the SRC-II processing of Powhatan No. 5 Mine coal on PDU P-99 at Harmarville, PA. 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 the recycle PS boiled over the range of 416-886°F and overlapped with the debutanizer bottoms boiling range. The only source of heavy distillate (HD) boiling above 900°F was atmospheric flash column bottoms, an internal process stream. A blend of the recycle PS and atmospheric flash bottoms in an appropriate ratio served as a full-boiling-range feedstock for distillation. The full-range material was fractionally distilled by Gulf Science and Technology Co. to produce the following bp cuts: 300-700°F, 700-750°F, 750-800°F, 800-850°F, and 850°F+ bottoms. The weight percentages of the distillate fractions relative to the source materials were recorded so that cuts could be rebled in the proper ratios to yield wider distillate range materials. A detailed description of the source materials and distillation methods, along with physical, chemical and thermodynamic properties of the resultant materials is found elsewhere (Gray, 1981).

EDS

The EDS sample set consisted of materials that had been fractionally distilled into 50°F cuts from a 60:40 volume percent blend of the raw naphtha and recycle solvent collected during processing of Illinois No. 6 coal in the bottoms recycle mode. Both product blending and fractional distillations were performed by Exxon Research and Engineering Co., Baytown, TX. The blended starting material was considered to be representative of a full-boiling-range product from the EDS process. In general, correlated chemical and biological

studies were conducted only on those distillates with bp ranges greater than 650°F.

H-COAL

H-Coal materials used in this study to formulate a blended material for fractional distillation were obtained from the Oak Ridge National Laboratory Synfuels Repository. Documentation accompanying the oils indicated that they were from 100-gallon samplings taken on October 30, 1981 from the Catlettsburg, KY plant during the processing of Illinois No. 6 coal in the syncrude mode. A light oil (N-203 light oil, PNL Sample No. 3399-146) and the heavy oil (N-202 heavy oil, PNL Sample No. 3399-147) were blended in a 1.4:1.0 heavy oil:light oil ratio; this blend was representative of a full-boiling-range syncrude material (boiling above 350°F). The blend was forwarded to Chevron Research Co, Richmond, CA, for distillation into the appropriate 50°F narrow-boiling fractions (Wilson et al., 1985).

ITSL

Samples from the C.E. Lummus ITSL process used in this study were collected during normal operation of the New Brunswick, NJ PDU facility with Indiana No. 5 coal. The ITSL process was configured so that the LC finer feed (first-stage product) was recycled once before entering the catalytic hydrogenation LC finer unit (second stage). To obtain data on biological activity and chemistry as a function of bp, the TLP from the LC finer was distilled into nominal bp cuts according to temperatures used in distillation mechanisms within the process. (These crude plant distillations may not be as discrete as the laboratory distillations of the other process materials; carryover of higher-boiling materials may be present in the lower-boiling fractions.) Although these distillate fractions had bp ranges wider than the 50°F cuts used for other samples, nevertheless, they served to provide the necessary biological activity data with respect to the 700°F cut point that had been obtained for distilled materials from the other four processes.

METHODS

CHEMICAL ANALYSIS

The various bp range materials were chemically analyzed and prepared for biological testing using an integrated analytical approach based on separation according to chemical class. This method was developed at PNL for the qualitative and quantitative measurement of components in complex coal liquefaction materials. It includes an initial fractionation by adsorption column chromatography, followed by high-resolution gas chromatography (HRGC), gas chromatography/mass spectrometry (GC/MS) and low-voltage probe-inlet mass spectrometry (LVMS). Selected chemical class fractions were further separated by high-performance liquid chromatography (HPLC) prior to HRGC and MS analyses. Details of the methods employed are outlined below.

Adsorption Column Chromatography

Samples outlined in the Sample Materials section were fractionated by chemical class using adsorption column chromatography methods developed specifically for the preseparation of coal-derived materials by Later et al. (1981; Later and Lee, 1983). Samples were sequentially eluted from standardized alumina with hexane, benzene, chloroform:ethanol (99:1), and methanol to produce aliphatic hydrocarbon (AH), PAH, NPAC, and hydroxy-substituted PAH (hydroxy-PAH) chemical class fractions. The NPAC species were further separated on standardized silicic acid using hexane, hexane:benzene (1:1), and benzene:ethyl ether (1:1) eluents to produce carbazole, enriched amino-PAH, and azaarene subfractions. The amino-PAH isolate was then derivatized with pentafluoropropionic anhydride (PFPA) for selective qualitative and quantitative analysis by HRGC with an electron capture detector (ECD; Later et al., 1982). Details of these separation schemes are shown in Figure 6.

High-Resolution Gas Chromatography (HRGC)

The neutral PAH, NPAC and amino-PAH fractions of the bp range materials were analyzed by HRGC using a Hewlett-Packard (HP) 5880 gas chromatograph

FIGURE 6. Adsorption Column Chromatography Separation Scheme for Chemical Class Fractionation

equipped with either a 15 m or a 25 m x 0.25 mm ID fused silica capillary column coated with SE-54 or DB-5 (0.25 μ m film thickness; J & W Scientific). Samples were introduced at 275°C in the splitless mode with helium as the carrier gas at 50 cm/sec linear velocity. In general, the oven temperature was held for 2 minutes at 50°C, then temperature-programmed to 300°C and held at the final temperature for 5 minutes. Exact parameters are given in appropriate figure legends. A flame ionization detector (FID) was used to analyze PAH and NPAC fractions. A nitrogen-phosphorus detector (NPD) was used in conjunction with the FID to verify that the NPAC components eluted in the proper fraction. A ^{63}Ni ECD was used for the analysis of PFPA derivatized amino-PAH. All detectors were operated between 285 and 300°C.

Fraction components were identified by retention index (Lee et al., 1979; Vassilaros et al., 1982) and absolute retention times of standard polycyclic aromatic compounds (PAC). Quantitative analyses of EDS, H-Coal and ITSL bp range materials were performed using an internal standard (ISTD) calibration method, as described by Wilson et al. (1984). 2-Chloroanthracene was added as an ISTD to all fractions at a final concentration of 50 ng/ μ l. Response factors were calculated from standard PAC chromatographed at eight concentration levels ranging over two orders of magnitude. A response factor of a closely eluting or similar compound was used to quantify components for which standards were not available. An external standard method using standard PAC was used for the SRC-I and -II bp range materials. All fractions were analyzed at two or more dilutions ranging from 1.0 to 10.0 mg/ml to ensure that analyses were within the range of the calculated response factors. Averaged results are reported in parts per million (ppm), μ g/g, or in parts per thousand (ppt), mg/g, as noted for a particular fraction.

Gas Chromatography/Mass Spectrometry (GC/MS)

Retention index and absolute retention time identifications were supplemented with coupled GC/MS determinations. The GC/MS analyses were performed on an HP-5982A quadrupole mass spectrometer interfaced with an HP-5710 GC equipped with a 15 m x 0.30 mm ID, DB-5 fused-silica capillary column. The GC transfer line was modified so that the fused silica could be inserted to within 5 to 10 mm of the ionizing electron beam within the ion source. This

modification enhanced the resolution and interpretability of the GC/MS chromatograms. Gas chromatographic conditions were similar to those previously described for GC analyses. The quadrupole MS was operated in the electron impact mode at 70 eV, and scan rates were typically 100 atomic mass units per second (amu/sec). Spectra of the capillary GC peaks were acquired and processed on an HP-5934A data system. Identifications were assigned to sample components, based on their similarity with mass spectra in the Environmental Protection Agency (EPA)/National Institutes of Health (NIH) library or with spectra of authentic reference compounds run under identical GC/MS conditions.

Low-Voltage Probe-Inlet Mass Spectrometry (LVMS)

LVMS was used as an additional means of identifying compounds present in selected crudes and fractions of the bp range coal liquids (as outlined in the Sample Materials section). This method was particularly useful in the analysis of materials not amenable to GC because of their low volatilities or highly polar natures. A VG ZAB 1-F double-focusing mass spectrometer was operated in the electron impact mode, with an accelerating voltage of 6000 or 7000 V, a magnet scan rate of 2 to 3 seconds/mass decade, a source temperature of 250°C, and a dynamic resolving power (as determined by the VG 2035 data system) of 1:2000.

Each sample (10 to 20 µg) was loaded into a glass capillary tube, which was inserted into the source affixed to the end of a direct insertion probe. The probe was heated in a linear fashion from ambient to 250-280°C, slowly desorbing the sample from the capillary tube in what amounted to fractional distillation. The mass spectrometer was scanned repetitively throughout this desorption period, and data were collected across the entire desorption profile. By using the data system to sum the intensities of each mass across the entire profile, an average spectrum was generated, representing the entire sample. These averaged spectra were then used to estimate the mean molecular weight of each sample and were compared to GC data to confirm the rank order of the average molecular weights assigned to the various samples.

Averaged spectra were acquired using ionizing electron energies of 10-12 eV; these spectra were composed primarily of signals representing molecular

ions rather than fragments or doubly charged ions. The resulting profile, therefore, more accurately represented the molecular weight distribution and composition of the sample as compared to the 70-eV spectra.

High-Performance Liquid Chromatography (HPLC)

The PAH fractions of selected bp range (°F) materials, including SRC-II 800-850 and 850+; EDS 650-700, 700-750, 750-800 and 800+; and ITSL 850+ distillate of the TLP, were further separated by reverse-phase HPLC according to the method of Lucke et al. (1985). A Spectra-Physics 8100 liquid chromatograph, equipped with a Waters Associates 440 ultraviolet (UV) absorbance detector fixed at a wavelength of 254 nm, was operated in the reverse-phase mode using a Supelcosil RP8-DB, 5 µm, 25 cm x 10 mm ID column. The mobile-phase composition and solvent gradient were optimized to provide the best possible separation for each individual 50°F distillate fraction. Typically, the eluent was a mixture of methanol (spectral grade, Burdick & Jackson) in water (doubly distilled, deionized Milli Q grade), with the methanol concentration gradient linearly programmed from approximately 70 up to 100% after an initial isocratic period. Subfractions of the neutral PAH fractions were collected for biological and chemical analysis according to schemes similar to that shown in Figure 7.

BIOLOGICAL ASSAYS

The biological activity of the bp range coal liquid materials was evaluated using four different assays. The mutagenic potencies for the crude materials and their chemical class fractions were estimated using the standard plate incorporation microsomal assay method of Ames et al. (1975). The tumor-initiating potencies of selected samples were evaluated by the I/P assay for mouse skin tumorigenicity. The genotoxicity of selected samples toward mammalian cells was assessed using a Chinese hamster ovary (CHO) mutation assay. Finally, the ecotoxicity of selected samples was evaluated in a static aquatic test system with Daphnia magna. The specific details of each of the bioassays are outlined below.

FIGURE 7. HPLC Separation of the Neutral PAH Isolate of the SRC-II 800-850°F Distillate Fraction

Standard Microbial Mutagenicity Test

Standard agar plate mutagenicity assays were performed as described by Ames et al. (1975) using Salmonella typhimurium, TA98 microbial tester strain. Dimethylsulfoxide (DMSO) was used as a solvent with Aroclor-induced rat liver (S9) homogenates used for metabolic activation. Optimum levels for S9 were established and monitored daily during the course of the experiments. Negative controls included the Salmonella test strains with and without microsomal enzymes (S9), and appropriate solvent controls. Revertant colonies per petri plate were counted electronically using a Biotran II automated colony counter.

The specific mutagenic activity of crude products and chemical fractions of the sample materials were expressed as revertant colonies of S. typhimurium, TA98 per μg of test material, estimated by linear regression analysis of dose response data. A positive test was one that had a maximum mutagenic response in revertant colonies that was twofold (or greater) above background. Mutagenic potency was estimated by linear regression analysis of dose response data. (In addition to the mutagens used to check the phenotypes of Salmonella strains, fixed concentrations of 2-aminoanthracene (2-AA), benzo[a]pyrene (BaP) and a complex basic fraction from SRC-II HD were tested as controls.)

The following criteria were used for selecting the best dose range for estimating a linear dose response: at least a four-point dose range; approximate doubling of response for doubled dose concentration; a correlation coefficient of 0.8 or greater; and an intercept on the response (ordinate) axis within 20% of the negative control for the day. Mutagenicity experiments were generally replicated several times to obtain an average value for the slope of the regression line, or mutagenic potency in revertants/ μg . Specific mutagenic activity is expressed as revertant colonies of TA98/ μg of test material. Maximum responses (given in revertants/plate) are also given. Control responses were consistent with historical values of 48 ± 16 and 53 ± 19 revertants/plate for less than 100 μl S9 (354 values) and 100 μl S9 added (120 values), respectively.

I/P Assay for Mouse Skin Tumorigenicity

The I/P mouse skin tumor assays were performed as described by Mahlum (1983a). Female CD-1 mice (Charles River Laboratories, Portage, MI), approximately 6 to 8 weeks of age, were housed five per cage on standard bedding material (Sani-Cel, Paxton Processing Co., Paxton, IL), with food and water available ad libitum. Prior to administration of test materials, the animals were ear-tagged for unique identification, weighed, shaved, and assigned to a test group, 30 animals per group. Each test material was diluted 1:1 with acetone or methylene chloride, and 50 μ l of the diluted material was applied to the shaved backs of the mice. The resulting dose was approximately 25 mg per mouse. (See Results and Discussion section for exact dosing of each material investigated.) Positive controls consisted of 50 μ g of BaP (Sigma Chemical Company); negative controls received 50 μ l of the appropriate solvents. Two weeks after initiation, 5- μ g doses of phorbol myristate acetate (PMA; Sigma Chemical Co.; 0.1 mg/ml of acetone) were applied to the initiated area, twice weekly for 24 weeks. The mice were shaved as necessary throughout the study, usually weekly. Animals were observed regularly for tumor growth, and the number of tumors per animal was counted biweekly. For comparison, tumors have been expressed as the total tumors per group of 30 mice.

The following selected crude bp range ($^{\circ}$ F) coal liquid samples were analyzed in the I/P assay: SRC-I, 550-600, 600-650, 650-700, 700-750, 750-800 and 800+; SRC-II, 300-700, 700-750, 750-800, 800-850 and 850+; EDS, 750-800 and 800+; H-Coal, 650-700, 700-750, 750-800, 800-850 and 850+; and the ITSL 850+ distillate of the TLP. Results for solvent controls, positive standard compounds and other coal- and petroleum-derived fossil fuels tested in the I/P assays are also presented for comparison.

CHO Cell Mutation Assay

The genotoxicity of SRC-I and -II bp range coal liquids and the ITSL 850 $^{\circ}$ F+ distillate of the TLP were evaluated using the CHO mammalian cell assay. A well-characterized assay system for measuring mutation frequency in these cells has been described elsewhere (O'Neill et al., 1977). Necessary modifications of those assay procedures included the use of activating enzymes

prepared from rat liver homogenates to detect compounds that required metabolic activation, and the use of six-well Linbro® plates (Mahlum et al., 1983).

Each monolayered culture contained approximately 1×10^6 exponentially growing cells. Cells were rinsed with saline, and graded doses of test materials were added, both with and without activating enzymes. Cells were incubated at 37°C for 5 hours. A typical experiment consisted of controls (positive, negative and solvent), as well as three doses of test compounds. Triplicate samples were tested for each dose.

Following exposure, cultures were rinsed, and cells were removed with trypsin. They were then washed to remove trypsin, counted, and 150 cells/well (six-well Linbro® chamber) were plated to determine cell survival. Remaining cells ($\sim 1 \times 10^6$) were plated in growth medium and incubated at 37°C. These cultures were transferred and diluted back to 1×10^6 cells at 2-day intervals. After an 8-day expression period, cells were again trypsinized, washed and counted. The cloning efficiency and mutation frequencies were then determined.

To obtain the cloning efficiency, each flask of cells was diluted, and one six-well plate was seeded with 150 cells; another plate was seeded with 300 cells/well. Cells were in 4 ml of hypoxanthine-free growth medium.

For mutant selection, each well (of the six-well plate) was seeded with 50,000 cells in 4 ml of selective medium (containing 6 µg/ml of 6-thioguanine). A total of 1.2×10^6 cells (24 wells) were screened for each data point. Plates were incubated at 37°C in a 100% humidified incubator in a 5% CO₂:95% air atmosphere. Dishes were fixed and stained (dilute crystal violet) after 7 days. Colonies were counted, and mutation frequency was calculated by dividing the total number of mutant colonies by the number of cells/plate, corrected for the cloning efficiency.

Aquatic Toxicity Assay

Ecotoxicity screening assays were also conducted with bp range materials from SRC-II, EDS and H-Coal processes. Acute toxicity of the water-soluble fractions (WSF) derived from the test materials was determined by conducting static bioassays with the freshwater invertebrate D. magna. All tests were conducted at 20°C with a 16:8-hour light:dark cycle. The test consisted of placing five first-instar D. magna in each of four 100-ml-capacity glass jars at six treatment levels, plus a control (4 x 7 matrix, 20 organisms per treatment level).

The WSF were generated for the bp range coal liquids by adding 100 ml of each sample material to 10 L of well water (1:100 ratio) in a 15-L-capacity glass carboy. The mixture was stirred at 90 rpm with a teflon-coated rod for 4 hours. After a 1-hour settling period, 8 L of the WSF were siphoned from the center of the carboy, chemically analyzed, and used for bioassays. Exposure solutions for testing were made by diluting WSF with well water.

Total carbon (TC) of the WSF was determined by direct aqueous injection into a Beckman 915B carbon analyzer. Detailed chemical analysis of the WSF by GC was conducted to determine phenol and hydrocarbon composition according to methodology outlined by Dauble et al. (1982, 1983). Total nitrogen base concentrations were determined by GC with a nitrogen-specific detector (Thomas, 1984).

Paired tests were conducted 1 week apart using a WSF generated from each of the three bp cuts derived from a single parent material. There was insufficient material available from all of the reconstructed cuts to generate additional WSF needed for true replication of test results. The 48-hour LC₅₀ determinations were made by the graphical method (American Public Health Association [APHA], 1976). Toxicity thresholds were expressed as percent WSF and TC, based on dilution of measured stock solution.

RESULTS AND DISCUSSION

In this section, a comparative summary of the chemical and biological data for the various bp distillate cuts from the SRC-I, -II, EDS, H-Coal and ITSL processes will be presented and discussed. This section will be divided into chemistry and biology subsections. Discussions dealing with both chemistry and biology data will be found in both subsections. Although 50°F bp cuts over the full-boiling range were available for most of the coal liquefaction processes considered in this Status Report, the majority of the data and discussion will focus on those materials with bp ranges greater than 700°F, since these materials were generally active in all the biological laboratory assays used in these investigations. Biological and chemical data for bp cuts with distillation temperatures below 700°F, for example 300-700°F or the 650-700°F, are presented to demonstrate the biological inactivity of coal liquefaction materials with bp ranges below the 700°F cut point.

CHEMICAL ANALYSES

Gross Chemical Properties

Inspection data for the SRC-I, -II, EDS, H-Coal and ITSL distillate fractions are provided in Table 2. The weight distributions for the H-Coal and EDS fractions were very similar, with more than 90% of the parent blend distilling below 700°F. Approximately 80% of the SRC-II feedstock was distillable in the range from the initial boiling point (IBP) to 700°F. Less than 41% of the SRC-I and approximately 25% of the ITSL materials were distilled at the 700°F mark, indicating that these distillate feedstocks were skewed toward heavier-end, higher-boiling materials.

Also provided in Table 2 are elemental analyses data for weight percent sulfur, nitrogen, oxygen, carbon and hydrogen. Several general trends in physical properties and elemental composition are apparent from these data. First, the °API gravity of the distillate cuts decreased with increasing distillation temperature. Second, the weight percent hydrogen decreased with increasing temperature. This implies a decreasing hydrogen:carbon ratio with

TABLE 2. Inspection Data for bp Cuts from the SRC-I, SRC-II, EDS, H-Coal, and ITSL Processes

Distillate	Wt%	°API	Weight Percent (Wt%)				
			Sulfur	Nitrogen	Oxygen	Hydrogen	Carbon
<u>SRC-I (Coal Type: Kentucky No. 9)</u>							
IBP-700	40.9	4.2 ^a					
700-750	6.7	2.0					
750-800	3.9	1.4					
800-850	5.5	-0.7					
850+	41.4	--					
	(98.4) ^b						
<u>SRC-II (Coal Type: Powhatan No. 5)</u>							
IBP-700	78.9	7.4	0.32	0.90	2.29	8.37	88.12
700-750	5.5	0.0	0.47	1.31	1.90	7.66	88.66
750-800	7.4	-2.2	0.36	1.39	1.81	7.34	89.10
800-850	3.5	-5.2	0.44	1.56	2.03	7.27	88.70
850+	4.7	-8.9	0.61	1.75	1.99	7.02	88.63
	(100.0) ^b						
<u>H-Coal (Coal Type: Illinois No. 6)</u>							
IBP-700	90.2	3.4	0.23	0.82	0.88	8.45	89.62
700-750	1.4	1.9	0.24	0.76	1.47	7.79	89.74
750-800	4.1	1.4	0.24	0.98	1.65	7.84	89.29
800-850	1.1	-2.4	0.24	1.25	0.96	7.48	90.07
850+	3.1	-13.3	0.39	1.80	2.73	6.77	88.31
	(99.9) ^b						
<u>EDS (Coal Type: Illinois No. 6)</u>							
IBP-700	91.4	4.6 ^a					
700-750	2.8	1.4					
750-800	2.2	0.6					
800+	3.6	-4.1					
	(99.0) ^b						
<u>ITSL Lummus (Coal Type: Indiana No. 5)</u>							
IBP-390	4.8	38.7	0.08	<0.20	0.99	13.02	85.11
390-500	5.3	22.2	0.07	<0.20	0.73	11.14	87.18
500-650	11.4	12.2	0.07	<0.20	0.32	10.09	88.81
650-850	32.7	0.2	0.20	0.31	0.53	8.42	91.23
850+	42.9	-16.1	0.88	1.71	4.66	5.40	85.71
	(97.1) ^b						

^a°API gravity given for 650-700°F bp cut

^bCumulative Wt%

increasing distillation temperature. The decrease in hydrogen:carbon ratio was paralleled by increasing heteroatom concentrations, indicating an increase in heteroatomic functional compounds in the higher-boiling distillate fractions. Elemental nitrogen, sulfur and oxygen all increased in concentration with increasing boiling point; maximum weight percentages for all three elements were found in the highest bp cuts, 800°F+ or 850°F+, for the three sets of materials shown.

Figure 8, taken from inspection data reported for the H-Coal (Wilson et al, 1985) and SRC-II (Gray, 1981) materials, graphically illustrates this observation. In both materials, heteroatom content increased with increased bp. The relatively high oxygen concentrations in the 300-500°F bp regions of the H-Coal and SRC-II materials were primarily due to the presence of phenols that distill in this temperature range. Nitrogen content generally increased with molecular weight or bp, as did sulfur. Above 700°F, in the case of both nitrogen and oxygen, the percentage of molecules in the H-Coal and SRC-II distillate cuts having at least one nitrogen or oxygen atom increased, since the overall molecular weight of the distillates increased with temperature. This implies that increases in the weight distribution of chemical classes of oxygen- and nitrogen-containing compounds would be expected with increasing distillation temperature. This is shown to be the case in the following discussion on chemical class composition. This same observation (increase of nitrogen and oxygen content with increasing distillation temperature) has also been noted for the SRC-I, EDS and ITSL materials.

Chemical Class Composition

The weight distributions for the four major chemical classes of compounds, as determined by alumina adsorption chromatography, are shown in Table 3. For distillate cuts boiling below the 700°F cut point, the AH compounds were generally the most concentrated chemical class, particularly when considering a broad-boiling-range sample such as an IBP-700°F cut. As was discussed in the previous section, higher hydrogen:carbon ratios were observed in lower-boiling distillate fractions. Hence, it would be expected that compounds in the lower bp cuts would contain higher levels of saturated hydrocarbons, particularly when the hydrogen:carbon ratio exceeded 2.0.

FIGURE 8. Distribution of Heteroatom Content in the H-Coal and SRC-II Distillation Fractions

TABLE 3. Chemical Class Weight Distribution as Determined by Adsorption Chromatography for the bp Distillate Cuts from the SRC-I, SRC-II, EDS, H-Coal and ITSL Processes

Fraction	Weight Distribution (%) ^a				
	<700°F	700-750°F	750-800°F	800-850°F	850°F+
SRC-I					
A ₁	35 ^b	27	18	5 ^c	
A ₂	30	31	30	20	
A ₃	18	21	23	36	
A ₄	9	10	14	35	
TOTAL	92	89	85	93	
SRC-II					
A ₁	32 ^d	15	15	4	2
A ₂	27	45	43	47	44
A ₃	12	21	29	31	35
A ₄	15	11	12	16	19
TOTAL	86	92	99	98	100
H-Coal					
A ₁	16 ^b	11	16	17	3
A ₂	55	59	50	45	40
A ₃	14	14	16	21	31
A ₄	15	16	18	20	26
TOTAL	100	100	100	103	100

^aWeight distribution data presented are the average values of three or more determinations

^bWeight distribution data given for 650-700°F bp cut

^cWeight distribution data given for 800°F+ bp cut

^dWeight distribution data given for 300-700°F bp cut

KEY:

A₁ = Aliphatic hydrocarbon

A₂ = Neutral PAH

A₃ = NPAC

A₄ = Hydroxy-PAH

TABLE 3. (Continued)

Fraction	Weight Distribution (%) ^a				
	<700°F	700-750°F	750-800°F	800-850°F	850°F+
EDS					
A ₁	23 ^b	19	16	4 ^c	
A ₂	52	60	58	58	
A ₃	10	9	13	23	
A ₄	8	9	12	17	
TOTAL	93	97	99	102	
ITSL ^e					
		500-650°F	650-850°F	850°F+	
A ₁		60	34	2	
A ₂		27	56	21	
A ₃		2	4	29	
A ₄		<1	11	45	
TOTAL		90	105	97	

^aWeight distribution data presented are the average values of three or more determinations

^bWeight distribution data given for 650-700°F bp cut

^cWeight distribution data given for 800°F+ bp cut

^dWeight distribution data given for 300-700°F bp cut

^eNon-laboratory-distilled samples (i.e., process-distilled samples) from pilot plant origins

KEY:

A₁ = Aliphatic hydrocarbon
A₂ = Neutral PAH
A₃ = NPAC
A₄ = Hydroxy-PAH

Considering the distillate cuts boiling above 700°F, the neutral PAH chemical class was found to be the most concentrated, with marked drop-offs in the AH levels. Generally, the neutral PAH concentration levels remained approximately constant for the high-boiling distillate fractions from all of the processes shown in Table 3. For example, the PAH fraction, A₂, had a range of only 43-47 weight percent between the 700-750°F bp cut to the 850°F+ fractions. Materials from those processes (ITSL, EDS and H-Coal) that employed

some form of catalytic hydrogenation had higher levels of neutral PAH, generally greater than 50%, than the noncatalytic SRC process, which generally had less than 50% PAH.

Finally, concentrations of the NPAC and hydroxy-PAH chemical classes increased in the distillate materials from all liquefaction processes in conjunction with increasing bp temperature. This trend was predicted from the elemental analysis data in the previous section. In the highest-boiling fractions of all process materials, the AH fractions were the least concentrated chemical class at nominally less than 5%. The major chemical species were the PAH and their heterofunctional nitrogen-, sulfur- and oxygen-derivative species. In general, polar oxygen- and nitrogen-containing compounds were most concentrated in the highest bp cuts, 800 or 850°F+, with nominal concentration ranges of greater than 20%.

Qualitative and Quantitative Analyses of Chemical Constituents

In-depth chemical characterizations were performed on the PAH and NPAC fractions isolated from the fractionally distilled coal liquefaction materials since these chemical class fractions had previously been shown to be the most mutagenic and/or tumorigenic in other coal-derived materials (Later et al., 1983a; Mahlum et al., 1984; Mahlum et al., 1983; Wilson et al., 1984). The results from GC, GC/MS and HPLC analyses of these two fractions follow. In addition, results of direct probe-inlet LVMS for the polar hydroxy-PAH fractions will be presented.

PAH Fractions. Figure 9 shows an example of the gas chromatograms obtained from HRGC analyses of the PAH fractions isolated from the 50°F distillate cuts above 700°F from the SRC-II materials. Similar sets of chromatograms have been reported for the 50°F distillate cuts from the greater than 650°F EDS (Later et al., 1984), greater than 650°F H-Coal (Wilson et al., 1985), greater than 700°F SRC-I, and the 850°F+ (process-distilled) ITSL TLP coal liquefaction process materials. In all cases, even though the materials were distilled into discrete bp range fractions, overlapping of chemical constituents in adjoining fractions occurred. Baseline resolution was not

FIGURE 9. Capillary Column Gas Chromatograms of the PAH Fractions Isolated from the SRC-II 50°F Distillate Cut Materials. Conditions: 50-100°C at 10°C/min; 100-300°C at 3°C/min.

achieved by HRGC for any of the PAH fractions of the 50°F bp cuts above 650°F due to the complexity of chemical constituents present.

The quantitative determinations for selected components in the PAH fractions, as determined by HRGC and GC/MS, for the EDS and H-Coal 650-700°F materials are given in Table 4. Similar compounds were present in both distillates. Catacondensed three-ringed and their alkylated homologs, and pericondensed four-ringed PAH compounds were the major components. The average molecular weight of the EDS material was determined to be 225 amu, and that of the H-Coal material was determined to be 220 amu by LVMS. Pyrene and dihydrofluoranthene were the most concentrated species at nearly identical levels in both materials. 2-Phenylnaphthalene was present at a significantly higher concentration in the H-Coal versus the EDS material.

Tables 5 and 6 present the quantitative determinations for selected components in the 700-750°F and 750-800°F, respectively, PAH fractions isolated from the SRC-I, -II, H-Coal and EDS materials. The 700-750°F distillate materials contained PAH ranging from pericondensed four rings, plus alkylated homologs, to catacondensed four rings. The average molecular weight, as determined by LVMS, was 235 amu for both the EDS and H-Coal 700-750°F distillate cuts. Pyrene, followed closely by benzo[b]fluorene or 2- or 4-methylpyrene, was the component of highest concentration in the SRC-II and H-Coal materials. A component of m/z 218, tentatively identified as a C₁-phenylnaphthalene or dihydro-216 (possibly a benzophenalanone), was the component of highest concentration in the SRC-I and EDS materials. The highest ratio of dihydrofluoranthene to fluoranthene was in the EDS material, as was the ratio of 1-methylpyrene to pyrene.

Alkylated peri- to alkylated catacondensed four-ringed PAH compounds were present in the 750-800°F distillate cut materials. Benzo[b]fluorene (or 2- or 4-methylpyrene) was the component of highest concentration in the SRC-II and H-Coal materials for this distillate range. Chrysene was the component of highest concentration in both the EDS and SRC-I materials. The average molecular weights for the EDS and H-Coal 750-800°F distillate cuts were 260 and 245 amu, respectively. The amounts of 6- or 4-methylchrysene and 1-methylpyrene were equal to or greater than the concentrations of their respective parent

TABLE 4. Quantitative Determinations of Selected Components in PAH Fractions of the H-Coal and EDS 650-700°F Distillate Cut Materials

Molecular Weight	Compound ^a	Concentration (mg/g PAH Fraction)	
		H-Coal	EDS
180	1-Methylfluorene	0.366 ± 0.107	ND ^b
178	Phenanthrene	1.16 ± 0.34	0.800 ± 0.086
192	3-Methylphenanthrene	6.54 ± 2.22	3.90 ± 0.48
192	2-Methylphenanthrene	13.3 ± 4.2	6.60 ± 0.70
192	1-Methylphenanthrene	5.00 ± 1.59	2.83 ± 0.25
204	2-Phenylnaphthalene	22.2 ± 5.2	6.00 ± 0.62
204	Dihydrofluoranthene	33.3 ± 11.1	ND
202	Fluoranthene	9.53 ± 2.58	9.80 ± 0.90
202	Pyrene	30.0 ± 8.1	30.0 ± 2.2
204	Dihdropyrene	4.55 ^c	ND

^aIdentifications based on GC/MS and retention times

^bNo quantitative data or none detected

^cOne determination only

compounds for the EDS materials; this was not the case for the SRC and H-Coal materials (see Tables 5 and 6).

The quantitative determinations for selected components in the PAH fractions for the SRC-II and H-Coal 800-850°F and the SRC-I and EDS 800°F+ distillate materials are given in Table 7. Alkylated four-ringed to five-ringed PAH compounds were the major constituents of these materials. 6- or 4-Methylchrysene was the component of highest concentration in the SRC-II and H-Coal materials. Distillate material boiling above 850°F was present in the EDS and SRC-I materials. This resulted in increased concentration of the benzo[fluor]anthrenes, benzopyrenes and benzo[ghi]perylene relative to the methylchrysenes compared to the SRC-II and H-Coal materials. This difference was further indicated by the average molecular weight LVMS determinations for the EDS 800°F+ and the H-Coal 800-850°F: 320 and 275 amu, respectively.

TABLE 5. Quantitative Determinations of Selected Components in PAH Fractions of the 700-750°F Distillate Cut Materials

Molecular Weight	Compound ^a	Concentration (mg/g PAH Fraction)			
		SRC-I	SRC-II	H-Coal	EDS
192	2-Methylphenanthrene	ND ^b	4.06 ± 1.00	0.117	ND
204	Dihydrofluoranthene	0.961 ± 0.140	21.8 ± 6.33	0.549 ± 0.027	ND
202	Fluoranthene	6.33 ± 0.84	30.2 ± 1.8	1.81 ± 0.02	1.10 ± 0.02
202	Pyrene	39.2 ± 5.9	276 ± 32	99.5 ± 3.4	30.0 ± 1.0
204	Dihdropyrene	10.7 ± 1.7	27.7 ± 8.4	7.68 ± 0.20	ND
216	Benzo[b]fluorene, 2- or 4-methylpyrene	103 ± 7	258 ± 70	83.0 ± 9.4	47.0 ± 12.0
218	C ₁ -phenylnaphthalene or dihydro 216	106 ± 16	104 ± 27	41.5 ± 0.1	55.0 ± 11.0
216	1-Methylpyrene	ND	34.3 ± 4.4	5.78 ± 2.50	27.0 ± 6.4
228	Benzo[a]anthracene	4.42	4.10 ± 0.94	0.835 ± 0.121	0.300 ± 0.023
228	Chrysene	1.05	2.09 ± 0.56	1.88 ± 0.14	1.60 ± 0.34
242	6- or 4-Methylchrysene	ND	0.165 ^c	0.546 ± 0.318	ND

^aIdentifications based on GC/MS and retention times

^bNo quantitative data or none detected

^cOne determination only

TABLE 6. Quantitative Determinations of Selected Components in PAH Fractions of the 750-800°F Distillate Cut Materials

Molecular Weight	Compound ^a	Concentration (mg/g PAH Fraction)			
		SRC-I	SRC-II	H-Coal	EDS
202	Fluoranthene	0.078 ± 0.007	2.37 ± 0.11	ND ^c	0.041 ^b
202	Pyrene	0.231 ± 0.028	32.8 ± 2.3	7.51 ± 0.07	1.20 ± 0.02
204	Dihdropyrene	ND	ND	0.406 ± 0.006	ND
216	Benzo[b]fluorene, 2- or 4-methylpyrene	6.11 ± 0.35	68.8 ± 8.0	54.5 ± 3.3	2.60 ± 0.23
218	C ₁ -phenylnaphthalene or dihydro 216	2.98 ± 0.47	23.0 ± 3.0	20.4 ± 2.0	2.20 ± 0.31
216	1-Methylpyrene	ND	14.8 ± 1.4	5.03 ± 0.52	1.40 ± 0.10
228	Benzo[a]anthracene	4.33 ± 0.42	5.71 ± 0.31	2.61 ± 1.02	5.50 ± 2.00
228	Chrysene	11.0 ± 0.6	7.11 ± 0.17	11.4 ± 1.4 ^d	22.0 ± 13.0
242	6- or 4-Methylchrysene	3.55 ± 0.36	8.73 ± 0.64	6.03 ± 0.21	21.0 ± 13.0

^aIdentifications based on GC/MS and retention times

^bOne determination only

^cNo quantitative data or none detected

^dCo-eluted with component of m/z 244

TABLE 7. Quantitative Determinations of Selected Components in PAH Fractions of the 800-850°F and 800°F+ Distillate Materials

Molecular Weight	Compound ^a	Concentration (mg/g PAH Fraction)			
		SRC-I ^b	SRC-II ^c	H-Coal ^c	EDS ^b
202	Pyrene	ND ^d	2.38 ± 0.05	ND	0.170 ^e
216	Benzo[b]fluorene, 2- or 4-methylpyrene	ND	5.74 ± 0.19	ND	ND
228	Benzo[a]anthracene	0.387 ± 0.091	3.00 ± 0.28	2.35 ± 0.06	ND
228	Chrysene	0.563 ± 0.100	7.32 ± 0.60	5.83 ± 0.21	ND
242	6- or 4-Methylchrysene	3.27 ± 0.31	35.2 ± 5.0	33.4 ± 0.98	4.20 ± 0.29
252	Benzo[j or b]fluoranthene	2.32 ± 0.44	9.59 ± 2.60	4.34 ± 0.34	6.40 ± 1.10
252	Benzo[k]fluoranthene	1.24	3.97 ± 0.51	2.54 ± 1.02	0.920 ± 0.340
252	Benzo[e]pyrene	2.31 ± 0.15	6.14 ± 0.72	3.31 ± 0.67	3.60 ± 0.92
252	Benzo[a]pyrene	1.71 ± 0.25	3.53 ± 0.39	2.32 ± 0.12	2.20 ± 1.20
276	Indeno[1,2,3-cd]pyrene	0.428	ND	0.384 ± 0.032	0.680 ± 0.160
278	Dibenz[a,c or a,h]anthracene	ND	ND	ND	0.037
276	Benzo[ghi]perylene	0.366	ND	0.437 ± 0.033	1.80 ± 0.40

^aIdentifications based on GC/MS and retention times

^b800°F+ distillate

^c800-850°F distillate cut

^dNo quantitative data or none detected

^eOne determination only

Benzo[ghi]perylene was the component in highest concentration for the SRC-II and ITSL TLP materials boiling above 850°F (see Table 8). Concentrations of benzo[e]pyrene or benzo[j or b]fluoranthene followed as next highest components. Similar results were achieved for the H-Coal 850°F+ material (see Table 8), except that the 6- or 4-methylchrysene concentration levels were comparable to the benzo[ghi]perylene concentration levels. The average molecular weights of the ITSL TLP and H-Coal 850°F+ PAH fractions were 265 and 300 amu, respectively.

Data compiled in Tables 5 through 8 indicated that the SRC-II and H-Coal distillate cut materials were similar. The EDS materials appeared to be more alkylated and hydrogenated in nature than the SRC or H-Coal materials. In addition, the EDS materials appeared to have higher average molecular weights than the H-Coal materials of the same distillation range.

In general, initiation of mouse skin tumorigenicity by coal liquefaction materials has been associated with their neutral PAH constituency (Mahlum et al., 1983, 1984). Therefore, the neutral PAH isolates of selected distillate cut materials from the EDS (Later et al., 1984), SRC-II (Lucke et al., 1985) and ITSL processes were subfractionated by reverse-phase HPLC. Mutagenicity, tumorigenicity and further chemical analyses were conducted on the subfractions. Detailed GC, GC/MS and LVMS analyses were performed in an effort to identify the specific components in these HPLC subfractions; complete data have been presented elsewhere (Lucke, R. B. and D. W. Later, Integrated Separation and Analysis of Coal-Derived Materials: A Status Report, Pacific Northwest Laboratory, Richland, WA; in preparation).

A combined capillary gas chromatogram and low-voltage mass spectrum of the EDS 800°F+ HPLC Subfraction 2 of the PAH fraction is shown in Figure 10. Note the degree of separation achieved by the HPLC method, as well as the high level of mass focusing. In this example, several PAH isomers were detected by HRGC, however, the masses for the components in the subfraction as determined by LVMS are either the same or within a small mass window (± 10 amu). Similar results were achieved for all the HPLC subfractions of the SRC-II 800-850°F and 850°F+; EDS 650-700°F, 700-750°F, 750-800°F and 800°F+; and ITSL TLP 850°F+.

TABLE 8. Quantitative Determinations of Selected Components in PAH Fractions of the 850°F+ Distillate Materials

Molecular Weight	Compound ^a	Concentration (mg/g PAH Fraction)		
		SRC-II	H-Coal	ITSL TLP
228	Benz[a]anthracene	ND ^b	0.601 ± 0.043	0.405 ± 0.122
228	Chrysene	0.031 ^c	1.33 ± 0.16	2.60 ± 0.40
242	6- or 4-Methylchrysene	0.215 ± 0.042	6.27 ± 1.39	7.07 ± 1.31
252	Benzo[j or b]fluoranthene	2.36 ± 0.22	4.07 ± 0.59	23.2 ± 7.4
252	Benzo[k]fluoranthene	0.306 ± 0.025	2.32 ± 0.74	5.92 ± 1.35
252	Benzo[e]pyrene	5.76 ± 0.50	3.62 ± 0.21	26.9 ± 6.6
252	Benzo[a]pyrene	3.64 ± 0.39	3.90 ± 0.20	11.2 ± 2.6
252	Perylene	ND	ND	4.06 ± 0.74
276	Indeno[1,2,3-cd]pyrene	7.23 ± 2.20	3.54 ± 0.68	16.5 ± 2.8
278	Dibenz[a,c or a,h]anthracene	2.00 ± 1.16	1.32 ± 0.28	3.84 ± 0.98
276	Benzo[ghi]perylene	15.3	5.40 ± 0.40	27.2 ± 6.9
300	Coronene	1.07 ± 0.15	ND	ND

^aIdentifications based on GC/MS and retention times

^bNo quantitative data or none detected

^cOne determination only

FIGURE 10. Capillary Column Gas Chromatogram and Mass Spectrum (12 eV) of HPLC Cut #2 of the Neutral PAH Isolate of the 800°F+ EDS Distillate Cut

Fractionation by HPLC after adsorption column chromatography increased HRGC resolution and allowed for more accurate quantitative analyses to be performed than before fractionation. Table 9 presents the quantitative results, as determined by HRGC, of selected components in the most mutagenic and/or tumorigenic HPLC subfractions (#2) of the PAH fractions isolated from the SRC-II 800-850°F and EDS 800°F+ distillate materials. In both cases, the benzo[a]fluoranthenes and benzo[a]pyrenes were found in the highest concentration in these biologically active subfractions as compared to the other HPLC subfractions produced from these materials. The methylchrysenes were also present at significant levels; these compounds are known carcinogens. The BaP was completely concentrated in HPLC Subfraction 2 for the EDS material. However, BaP was detected at significant levels in both adjacent HPLC subfractions for the SRC-II 800-850°F material; 3.41 and 9.87 ppt were detected in HPLC Subfractions 1 and 3, respectively.

The highest concentrations of the benzo[a]fluoranthenes and benzo[a]pyrenes for the SRC-II and ITSL TLP 850°F+ distillate materials were found in HPLC Subfractions 1 and 2, respectively, of the PAH fraction (see Table 10). BaP was found at a concentration of only 4.14 ppt in the adjacent SRC-II 850°F HPLC Subfraction 2. More overlap of composition was detected for the ITSL TLP subfractionation: 23.7 ppt BaP were present in the subsequent HPLC Subfraction 3. In general, the HPLC subfractionation produced isolates containing two categories of components: parent PAH compounds of differing ring size and alkylated PAH components of varying molecular weight. Overall, HPLC subfractions contained increasing concentrations of more highly alkylated materials as their elution time increased.

NPAC. The major components of the NPAC fractions isolated from the H-Coal and EDS 650-700°F distillate cut materials were the methylcarbazoles (see Table 11). Azapyrenes and/or azafluoranthenes were the major components found in the NPAC fractions of the 700-750°F distillate cuts of the SRC and H-Coal materials (see Table 12). No azapyrene/fluoranthene compounds were detected in the EDS 700-750°F distillate cut NPAC fractions; the methylcarbazoles still represented the major nitrogen components in this distillate cut material. One or more of the isomers of benzocarbazole were detected in all 750-800°F distillate cut materials (see Table 13). Azapyrenes were also pres-

TABLE 9. Quantitative Determinations of Selected Components in Mutagenic and/or Tumorigenic HPLC Subfractions of the PAH Fractions Isolated from the SRC-II 800-850°F and EDS 800°F+ Distillate Materials

Molecular Weight	Compound ^a	Concentration (mg/g HPLC Subfraction #2)	
		SRC-II ^b	EDS ^c
216	Benzo[b]fluorene, 2- or 4-methylpyrene	2.87 ± 0.28	ND ^d
228	Benz[a]anthracene	5.28 ± 0.27	ND
228	Chrysene	3.84 ± 0.95	ND
242	3-Methylchrysene	60.9 ± 1.2	27.6 ± 3.1
242	6- or 4-Methylchrysene	ND	15.8 ± 2.6
252	Benzo[j or b]fluoranthene	91.1 ± 11.5	295 ± 66
252	Benzo[k]fluoranthene	14.8 ± 3.7	34.3 ± 8.2
252	Benzo[e]pyrene	46.0 ± 7.7	304 ± 67.5
252	Benzo[a]pyrene	12.0 ± 2.0	132 ± 30.7
252	Perylene	2.71 ± 0.46	9.3 ± 1.7

^aIdentifications based on GC/MS and retention times

^b800-850°F distillate cut

^c800°F+ distillate

^dNo quantitative data or none detected

ent at significant levels in the SRC and H-Coal materials. Table 14 contains the quantitative determinations for selected NPAC components in the 800-850°F and 800°F+ distillate materials. The benzocarbazoles were major components in all cases. The increased boiling range of the EDS 800°F+ NPAC distillate material was indicated by the presence of dibenzocarbazole.

Compositions of the NPAC fractions from the SRC and H-Coal materials proved to be similar, as was the case with the PAH fractions. Both aza compounds and carbazoles were present in significant concentration. Carbazoles only were the major components of the NPAC fractions from the EDS distillate cut materials. (ITSL TLP full-boiling-range materials showed similar results as the EDS distillate cut materials; i.e., carbazoles persisted in NPAC frac-

TABLE 10. Quantitative Determinations of Selected Components in Mutagenic and/or Tumorigenic HPLC Subfractions of the PAH Fractions Isolated from the 850°F+ Distillate Materials

Molecular Weight	Compound ^b	Concentration (mg/g HPLC Fraction) ^a	
		SRC-II HPLC #1	ITSL TLP HPLC #2
242	6- or 4-Methylchrysene	0.750	1.11
252	Benzo[j or b]fluoranthene	66.8	170
252	Benzo[k]fluoranthene	15.0	39.4
252	Benzo[e]pyrene	205	179
252	Benzo[a]pyrene	115	80.9
252	Perylene	22.0	26.8
276	Indeno[1,2,3-cd]pyrene	4.14	ND ^c
278	Dibenz[a,c or a,h]anthracene	2.56	ND
276	Benzo[ghi]perylene	12.9	ND

^aValues reported are the average of three determinations

^bIdentifications based on GC/MS and retention times

^cNo quantitative data or none detected

tions while aza compound concentrations were significantly reduced [Wilson et al., 1983].) Amino-PAH were found at significantly higher concentrations in the SRC and H-Coal materials compared to the EDS and ITSL materials as described in the next section.

Amino-PAH. Tables 15 through 19 summarize the results of quantitation by ECD using PFPA-derivatized amino-PAH fractions to determine the concentrations of selected amino-PAH in NPAC fractions isolated from the various distillate cut materials. Concentrations of amino-PAH tended to be an order of magnitude greater in the SRC-II and H-Coal materials as compared to the EDS materials. Components detected ranged from the aminobiphenyls, aminofluorenes, and aminophenanthrenes/anthracenes in the lower boiling distillate cuts to the amino-fluoranthenes/pyrenes and aminochrysenes in the higher distillate cuts. Concentrations of amino-PAH were not determined for the NPAC fraction isolated

TABLE 11. Quantitative Determinations of Selected Components in NPAC Fractions of the 650-700°F Distillate Cut Materials

Molecular Weight	Compound ^a	Concentration (mg/g NPAC Fraction)	
		H-Coal	EDS
179	Benzo[h]quinoline	ND ^b	0.280 ± 0.043
179	Acridine	0.385 ± 0.159	0.170 ± 0.015
179	Benzo[f]quinoline, phenanthridine	2.07 ± 0.68	0.850 ± 0.170
167	Carbazole	6.07 ± 1.36	4.10 ± 0.34
193	2- or 3-Methylbenzo[f]quinoline	14.0 ± 3.8	ND
181	1-Methylcarbazole	10.7 ± 3.8	0.510 ± 0.120
181	3-Methylcarbazole	47.5 ± 1.1	18.0 ± 2.3
181	2-Methylcarbazole	35.1 ± 2.3	14.0 ± 0.6
181	4-Methylcarbazole	18.1 ± 1.9	9.70 ± 0.69

^aIdentifications based on GC/MS and retention times

^bNo quantitative data or none detected

TABLE 12. Quantitative Determinations of Selected Components in NPAC Fractions of the 700-750°F Distillate Cut Materials

Molecular Weight	Compound ^a	Concentration (mg/g NPAC Fraction)			
		SRC-I	SRC-II	H-Coal	EDS
167	Carbazole	2.26 ^b	0.564	ND ^c	0.270 ± 0.019
181	3-Methylcarbazole	ND	ND	0.294 ± 0.035	0.670 ± 0.010
181	2-Methylcarbazole	ND	ND	0.099	0.650 ± 0.027
181	4-Methylcarbazole	ND	ND	ND	0.320 ± 0.033
203	2-Azafluoranthene	1.12	6.64	ND	ND
203	1- or 4-Azapyrene	1.56	29.4	25.7 ± 0.7	ND
217	Benzo[a]carbazole	ND	0.765	ND	ND

^aIdentifications based on GC/MS and retention times

^bOne determination only

^cNo quantitative data or none detected

TABLE 13. Quantitative Determinations of Selected Components in NPAC Fractions of the 750-800°F Distillate Cut Materials

Molecular Weight	Compound ^a	Concentration (mg/g NPAC Fraction)			
		SRC-I	SRC-II	H-Coal	EDS
203	2-Azafluoranthene	0.426 ^b	1.63	ND ^c	ND
203	1- or 4-Azapyrene	5.37	29.4	22.2 ± 0.3	ND
217	Benzo[a]carbazole	5.50	18.5	5.65 ± 1.91	ND
217	Benzo[b]carbazole	ND	ND	3.20 ± 1.5	ND
217	Benzo[c]carbazole	ND	ND	1.37 ± 0.08	31.0 ± 8.0

^aIdentifications based on GC/MS and retention times

^bOne determination only

^cNo quantitative data or none detected

TABLE 14. Quantitative Determinations of Selected Components in NPAC Fractions of the 800-850°F and 800°F+ Distillate Materials

Molecular Weight	Compound ^a	Concentration (mg/g NPAC Fraction)			
		SRC-I ^b	SRC-II ^c	H-Coal ^c	EDS ^b
203	2-Azafluoranthene	ND ^d	0.047 ^e	ND	ND
203	1- or 4-Azapyrene	ND	3.75	ND	ND
217	Benzo[a]carbazole	0.753	20.5	9.17 ± 1.51	ND
217	Benzo[b]carbazole	ND	ND	6.51 ± 0.85	ND
217	Benzo[c]carbazole	ND	ND	9.88 ± 1.81	0.400 ± 0.066
267	Dibenzo[a,g or c,g]-carbazole	ND	ND	ND	0.180

^aIdentifications based on GC/MS and retention times

^b800°F+ distillate

^c800-850°F distillate cut

^dNo quantitative data or none detected

^eOne determination only

TABLE 15. Quantitative Determinations of Selected Amino-PAH Components in NPAC Fractions of the 650-700°F Distillate Cut Materials

Molecular Weight ^b	Compound ^c	Concentration ^a (µg/g NPAC Fraction)	
		H-Coal	EDS
315	2-Aminobiphenyl	ND ^d	0.3
289	2-Aminonaphthalene	ND	0.3
293	3-Methyl-2-aminonaphthalene	28	2.2
315	4-Aminobiphenyl	44	10.3
327	4-Aminofluorene	570	25.0
327	2-Aminofluorene	764	ND
339	9-Aminophenanthrene ^e	ND	22.9

^aValues reported are the average of three determinations using an ECD and pentafluoropropylamide-derivatized amino-PAH fractions.

^bPentafluoropropylamide-derivatized mass as determined by GC/MS

^cIdentifications based on GC/MS and retention times

^dNo quantitative data or none detected

^eCo-elutes with 1-aminophenanthrene and 1-aminoanthracene

from the ITSL TLP 850°F+ distillate material. Results of analyses for the corresponding full boiling range material, however, indicated the presence of selected amino-PAH at approximately 15 ppm (Wilson et al., 1983). Hence, as with the EDS distillate materials, the amino-PAH concentration in the 850°F+ distillate of the ITSL material would also be lower than the H-Coal and SRC materials. The use of catalytic hydrogenation in the ITSL and EDS processes is felt to be responsible for the lower levels of amino-PAH in these materials.

Hydroxy-PAH. Results of LVMS hydroxy-PAH fraction analyses for the 750-800°F distillate cuts from SRC-II, H-Coal and EDS materials are shown in Figure 11. Possible structures corresponding to correct molecular weights and elemental compositions (as determined by high-resolution MS at 40 eV and 8000-9000 resolving power for the H-Coal 700-750°F distillate cut material,

TABLE 16. Quantitative Determinations of Selected Amino-PAH Components in NPAC Fractions of the 700-750°F Distillate Cut Materials

Molecular Weight ^b	Compound ^c	Concentration (µg/g NPAC Fraction) ^a		
		SRC-II	H-Coal	EDS
315	4-Aminobiphenyl	5.6	ND ^d	4.2
327	4-Aminofluorene	41.7	ND	15.5
327	2-Aminofluorene	593	174	80.7
339	9-Aminophenanthrene ^e	193	1460	194
339	2- or 3-Aminophenanthrene	624	2920	130
339	2-Aminoanthracene	573	2950	757
363	3-Aminofluoranthene	50.6	291	41.2
363	2-Aminopyrene	25.5	927	44.6
363	1-Aminopyrene	34.4	ND	14.8
363	8-Aminofluoranthene	9.5	ND	28.0
389	6-Aminochrysene	21.2	ND	ND

^aValues reported are the average of three determinations using an ECD and pentafluoropropylamide-derivatized amino-PAH fractions.

^bPentafluoropropylamide-derivatized mass as determined by GC/MS

^cIdentifications based on GC/MS and retention times

^dNo quantitative data or none detected

^eCo-elutes with 1-aminophenanthrene and 1-aminoanthracene

hydroxy-PAH fraction) are given; isomers of the structures postulated are possible. Distillate cuts above and below those shown contained similar hydroxy-PAH components only with more or less alkylation.

For any given bp cut, the average molecular weight of the hydroxy-PAH fractions of the EDS material tended to be higher than that of the SRC-II and H-Coal materials (in a similar fashion as that noted for the PAH fractions). For the 750-800°F distillate materials shown in Figure 11, the average molecular weights were 244 and 248 amu for the SRC-II and H-Coal hydroxy-PAH fractions, respectively. However, the average molecular weight of the EDS 750-800°F hydroxy-PAH distillate material was approximately 266 amu. Biphenyls

TABLE 17. Quantitative Determinations of Selected Amino-PAH Components in NPAC Fractions of the 750-800°F Distillate Cut Materials

Molecular Weight ^b	Compound ^c	Concentration (µg/g NPAC Fraction) ^a		
		SRC-II	H-Coal	EDS
327	2-Aminofluorene	143	ND ^d	7.2
339	9-Aminophenanthrene ^e	168	42	16.2
339	2- or 3-Aminophenanthrene	640	693	73.2
339	2-Aminoanthracene	371	1030	73.1
363	3-Aminofluoranthene	1420	899	389
363	2-Aminopyrene	475	ND	193
363	1-Aminopyrene	535	736	173
363	8-Aminofluoranthene	1090	863	157
389	6-Aminochrysene	61.5	62	33.1
389	3-Aminochrysene	119	ND	12.8

^aValues reported are the average of three determinations using an ECD and pentafluoropropylamide-derivatized amino-PAH fractions.

^bPentafluoropropylamide-derivatized mass as determined by GC/MS

^cIdentifications based on GC/MS and retention times

^dNo quantitative data or none detected

^eCo-elutes with 1-aminophenanthrene and 1-aminoanthracene

and phenylanthrols were found in increased concentration in the SRC-II and H-Coal hydroxy-PAH fractions as compared to the EDS hydroxy-PAH fractions of the bp range materials. Postulated structures for the hydroxy-PAH detected in all materials ranged from alkylated two-ringed hydroxy-PAH to alkylated four-ringed hydroxy-PAH.

BIOLOGICAL TESTING

Microbial Mutagenicity Assays (Ames Assay)

Table 20 provides a compilation of the microbial mutagenicity data for the crude distillates and their chemical fractions from the various direct

TABLE 18. Quantitative Determinations of Selected Amino-PAH Components in NPAC Fractions of the 800-850°F and 800°F+ Distillate Materials

Molecular Weight ^b	Compound ^c	Concentration (µg/g NPAC Fraction) ^a		
		SRC-II ^d	H-Coal ^d	EDS ^e
339	2- or 3-Aminophenanthrene	121	ND ^f	ND
339	2-Aminoanthracene	76.4	ND	ND
363	3-Aminofluoranthene	522	69	3.0
363	2-Aminopyrene	771	365	5.1
363	1-Aminopyrene	772	413	5.1
363	8-Aminofluoranthene	2130	3240	12.6
389	6-Aminochrysene	655	311	46.9
389	3-Aminochrysene	1030	404	20.3
413	7-Aminobenzo[a]pyrene	73.4	62	8.2

^aValues reported are the average of three determinations using an ECD and pentafluoropropylamide-derivatized amino-PAH fractions.

^bPentafluoropropylamide-derivatized mass as determined by GC/MS

^cIdentifications based on GC/MS and retention times

^d800-850°F distillate cut

^e800°F+ distillate

^fNo quantitative data or none detected

liquefaction processes. These data illustrate that, in general, the mutagenic activity of the unfractionated crude distillate cuts tended to increase with increasing distillation temperature in the Salmonella histidine reversion assay, regardless of the process technology. Similar results in each of the previous Status Reports have highlighted this observation. Furthermore, this trend was found to persist in a battery of other in vitro and in vivo assays (Mahlum et al., 1983). The peak mutagenic activity of the crudes was always either in the 800-850°F, 800°F+ or 850°F+ distillate fraction. The activity of some of the highest boiling crudes, notably the SRC-I 800°F+ and the H-Coal 850°F+ materials, did however, dip below the next lowest bp cut. This was most probably due to the drop-off in activity of the NPAC fraction and, in

TABLE 19. Quantitative Determinations of Selected Amino-PAH Components in NPAC Fractions of the 850°F+ Distillate Materials

Molecular Weight ^b	Compound ^c	Concentration ^a (µg/g NPAC Fraction)	
		SRC-II	H-Coal
363	8-Aminofluoranthene	53.9	ND ^d
389	6-Aminochrysene	34.0	56
389	3-Aminochrysene	119	177
413	7-Aminobenzo[a]pyrene	412	362

^aValues reported are the average of three determinations using an ECD and pentafluoropropylamide-derivatized amino-PAH fractions.

^bPentafluoropropylamide-derivatized mass as determined by GC/MS

^cIdentifications based on GC/MS and retention times

^dNo quantitative data or none detected

particular, the concentration levels of the three- to four-ringed amino-PAH in these higher boiling distillates.

Of the chemical fractions tested and shown in Table 20, the AH and the neutral PAH were almost always inactive in the Salmonella mutagenicity assay. Although it is not surprising that the AH fractions were inactive, putative mutagens such as BaP and the methylchrysenes were found in the neutral PAH fractions of these distillate cuts, as previously discussed in the chemistry section, and should have produced positive responses in this assay. The lack of significant activity in these fractions is felt to be due to interference effects of complex mixtures that mediate the expression of activity of this particular class of compounds in this assay.

Further mass-specific subfractionation by reverse-phase HPLC and microbial mutagenicity testing of the subfractions revealed that mutagens present in these coal-derived materials, such as BaP, could be detected against TA98. Figure 12 illustrates this approach for the SRC-II 850°F+ distillate cut. Al-

FIGURE 11. 12-eV Mass Spectra of Hydroxy-PAH Fractions Isolated from the SRC-II (Top), H-Coal (Middle), and EDS (Bottom) 750-800°F Distillate Cut Materials

TABLE 20. Microbial Mutagenicity of Distillate Fractions from the SRC-I, -II, EDS, H-COAL and ITSL Processes against S. typhimurium, TA98

Boiling Point (°F)	Dose Response in Revertants/μg: (Maximum Response in Revertants/Plate)									
	Crude	A ₁ : AH		A ₂ : PAH		A ₃ : NPAC		A ₄ : Hydroxy-PAH		
<u>SRC-I</u>										
600-650	0	(55 ± 3)	0 (40 ± 11)	0 (48 ± 15)	0	(71 ± 2)	0	(58 ± 5)		
650-700	6	(82 ± 12)	0 (69 ± 14)	0 (54 ± 10)	85	(298 ± 3)	0	(46 ± 4)		
700-750	13	(99 ± 35)	0 (39 ± 7)	0 (45 ± 4)	84	(242 ± 9)	0	(45 ± 13)		
750-800	34	(255 ± 16)	0 (70 ± 22)	0 (85 ± 16)	206	(655 ± 106)	0	(109 ± 10)		
800+	3	(218 ± 2)	0 (79 ± 18)	0 (59 ± 20)	31 ± 7	(161 ± 25)	4 ± 2	(114 ± 59)		
<u>SRC-II</u>										
300-700	0	(79 ± 2)	0 (36 ± 19)	0 (50 ± 13)	114 ± 5	(255 ± 96)	0	(73 ± 7)		
700-750	37 ± 14	(220 ± 0)	0 (42 ± 6)	0 (92 ± 5)	407 ± 271	(1713 ± 406)	0	(60 ± 31)		
750-800	67 ± 0	(524 ± 211)	0 (61 ± 4)	<1 (111 ± 16)	275 ± 46	(1455 ± 170)	0	(74 ± 24)		
800-850	61 ± 8	(943 ± 15)	0 (40 ± 8)	<1 (152 ± 43)	230 ± 20	(1908 ± 750)	1 ± 21	(272 ± 113)		
850+	258 ± 31	(1820 ± 56)	0 (68 ± 7)	1 (245 ± 41)	176	(2329 ± 37)	5 ± 2	(529 ± 180)		
<u>EDS</u>										
600-650	0	(79 ± 2)	0 (ND) ^a	0 (38 ± 0)	0	(49 ± 1)	0	(31 ± 1)		
650-700	0	(47 ± 3)	0 (50 ± 8)	<1 (139 ± 9)	0	(64 ± 3)	0	(45 ± 1)		
700-750	0	(60 ± 2)	0 (50 ± 5)	0 (68 ± 4)	13	(118 ± 6)	0	(45 ± 2)		
750-800	0	(103 ± 4)	0 (58 ± 8)	0 (98 ± 6)	34	(251 ± 5)	0	(53 ± 1)		
800+	34 ± 1	(189 ± 1)	0 (58 ± 2)	<1 (106 ± 55)	61	(362 ± 11)	0	(73 ± 9)		

^aND = no data

TABLE 20. (Continued)

Boiling Point (°F)	Dose Response in Revertants/μg: (Maximum Response in Revertants/Plate)								
	Crude	A ₁ : AH	A ₂ : PAH	A ₃ : NPAC	A ₄ : Hydroxy-PAH				
<u>H-Coal</u>									
650-700	0	(56 ± 2)	0 (69 ± 2)	0 (60 ± 3)	109	(374 ± 10)	0	(65 ± 1)	
700-750	65	(122 ± 5)	0 (60 ± 2)	0 (79 ± 0)	209	(1503 ± 87)	0	(81 ± 3)	
750-800	45	(155 ± 19)	0 (40 ± 1)	0 (81 ± 1)	184	(1397 ± 44)	3	(115 ± 13)	
800-850	104	(481 ± 10)	0 (60 ± 3)	0 (83 ± 0)	340	(2096 ± 71)	1	(377 ± 14)	
850+	70	(420 ± 51)	0 (55 ± 1)	<1 (201 ± 3)	140	(728 ± 22)	3	(2339 ± 16)	
IBP-850+	11	(325 ± 37)	0 (57 ± 6)	0 (61 ± 2)	652	(1923 ± 17)	2	(548 ± 12)	
<u>ITSL</u> ^b									
IBP-390	0	(73 ± 6)	0 (ND) ^a	0 (ND)	0	(59 ± 1)	0	(56 ± 14)	
390-500	0	(59 ± 3)	0 (41 ± 9)	0 (67 ± 4)	<1	(ND)	0	(61 ± 0)	
500-650	0	(81 ± 4)	0 (57 ± 2)	0 (58 ± 5)	74	(402 ± 7)	<1	(91 ± 3)	
650-850	48 ± 8	(62 ± 2)	0 (45 ± 2)	0 (55 ± 0)	793	(2163 ± 250)	1	(107 ± 2)	
850+	50 ± 34	(174 ± 0)	0 (56 ± 2)	0 (65 ± 13)	76	(414 ± 54)	5	(186 ± 23)	
IBP-850+ TLP	18	(637 ± 141)	0 (73 ± 4)	0 (71 ± 2)	124	(669 ± 4)	22	(471 ± 10)	

^aND = no data^bNon-laboratory-distilled samples, i.e., process-distilled samples from pilot plant origins

FIGURE 12. Induced Mutagenic Response of HPLC Subfractions from the Neutral PAH Fractions of the SRC-II 850°F+ Distillate Cut in the Salmonella Histidine Reversion Assay

though the unfractionated neutral PAH alumina fraction was inactive (see Table 20), from the results shown in Figure 12, mutagenic PAH constituents were obviously present in this fraction. Table 10 in the chemistry section provides a listing of the major constituents in HPLC Subfraction #1 of the SRC-II 850°F+ PAH fraction; BaP is one of the more prominent compounds.

Other variations of the microbial mutagenicity assay have also been employed to demonstrate the presence of active mutagens in the neutral PAH fractions of these materials (Mahlum et al., 1983). Additionally, the mutagenic activity of HPLC-subfractionated EDS bp cuts was discussed by Later et al. (1984). In all cases cited and shown here, the genetic activity of the neutral PAH isolates has been S9-dependent, suggesting that only promutagens were present in these fractions.

The majority of mutagenic activity occurred in the NPAC fractions, particularly in those distillate fractions with boiling temperatures above 700°F. Activities of the NPAC fractions were also dependent on distillation temperature. Overall, the activity increased to a maximum in the region of 700-800°F and then tailed off in the highest boiling fractions. This trend was observed for all the materials shown in Table 20. The H-Coal NPAC fractions actually demonstrated a bimodal character, with maxima in the 700-750°F and 800-850°F fractions.

As was noted earlier, the potency of the crude bp cuts generally correlated well with the activity of the NPAC fractions, suggesting that the NPAC constituents were the principal microbial mutagens in these materials. As was shown in the Chemical Analyses Section, the NPAC fractions contained significant levels of amino-PAH, particularly in those distillate materials that demonstrated the highest activities. Those materials that had been processed by some form of catalytic hydrogenation, namely the EDS and ITSL distillate fractions, generally contained lower levels of amino-PAH and were correspondingly less active in the TA98 microbial mutagenicity assay. The degree of activity expressed by the crude and the NPAC class fractions correlated with the concentration levels of amino-PAH. This observation has been presented in detail in all of the Status Reports dealing with these distilled coal liquids (Later

et al., 1984; Wilson et al., 1985; Mahlum et al., 1983a; Wilson et al., 1983a; Pelroy and Wilson, 1981; Wilson et al., 1982a).

The last alumina fraction, A₄, contained the hydroxy-PAH. The microbial mutagenicity of this fraction was also temperature-dependent but varied from one process to the other. For example, the highest boiling SRC-I, -II and ITSL distillates showed activity in the hydroxy-PAH fraction, while the EDS A₄ fractions were all inactive. The hydroxy-PAH fractions of the H-Coal distillates showed the same bimodal activity as the crudes and NPAC fractions.

For comprehensive compilations of the microbial mutagenicity assay data, in terms of dose-response and positive/negative controls, the reader is referred to the Appendices of the previously cited Status Reports on these various processes.

I/P Tumorigenicity Assay

Tumor-initiating activities of the distillate cuts from the SRC-I, -II, EDS, H-Coal and ITSL PDU processes are shown in Table 21. Since these I/P data were obtained from several different experiments, it is not totally appropriate to directly compare the results for the various sets of bp cuts. However, data from a given set of distillate fractions can be used to determine relative activities and define observable trends. In general, the I/P results are unanimous in indicating that skin tumor initiating activity of the distillate cuts from each set of process materials is a function of distillation temperature: an increase in initiating activity accompanies an increase in bp temperature.

As bp increased, both tumor incidence and total numbers increased for all materials studied. In all cases, the highest boiling distillate fraction from each process was the most potent skin tumor initiator. Tumor-initiating activity for distillate fractions with bp below 700°F are either relatively low, compared to higher-boiling fractions, or at the level of the solvent controls. Significant initiating activity (approximately twofold above the solvent control level) occurred in most cases above the 700°F cut point. The exception to this was the <650°F ITSL distillate, which showed surprisingly high tumor

TABLE 21. Tumor-Initiating Activity of Coal Liquid Distillates from the SRC-I, -II, EDS, H-Coal and ITSL Processes

Boiling Point (°F)	Incidence (%) ^a	Total Tumors ^b
<u>SRC-I^c</u>		
550-600	30	8 (3)
600-650	15	7 (3)
650-700	18	10 (4)
700-750	44	13 (5)
750-800	78	38 (16)
800+	94	93 (39)
IBP-800+ (undistilled process solvent)	83	62 (26)
BaP (50 µg)	93	240 (100)
Solvent control	4	5 (2)
<u>SRC-II^d</u>		
300-700	17	12 (5)
700-750	36	14 (6)
750-800	34	12 (5)
800-850	63	34 (15)
850+	96	220 (95)
300-850+	64	31 (13)
BaP (50 µg)	86	232 (100)
Solvent control	17	16 (7)

^aPercentage of mice with tumors in a group of 30 animals

^bTotal number of tumors normalized to a group of 30 animals; values in parentheses are normalized as a percent of BaP response

^c1:1 dilution of coal liquid with acetone; 50 µl initiating dose applied $\cong$ 25 mg

^d850°F+ tested separately from other bp cuts; all tested at 25 mg equivalent dose

^e1:1 dilution of coal liquid with acetone; 50 µl initiating dose applied $\cong$ 25 mg

^fDiluted at 0.55/ml and 50 µl dose applied

^gNon-laboratory-distilled materials, i.e., process-distilled material from pilot plant origins; 1:1 dilution of coal liquid with acetone or MeCl₂; 50 µl initiation dose applied $\cong$; different experiments

TABLE 21. (Continued)

Boiling Point (°F)	Incidence (%) ^a	Total Tumors ^b
<u>EDS^e</u>		
750-800	70	52
800+	100	154
400-1000 (undistilled process solvent)	72	25
Solvent control	10	3
<u>H-Coal^f</u>		
650-700	13	4 (3)
700-750	30	14 (11)
750-800	66	35 (28)
800-850	86	68 (54)
850+	89	94 (75)
IBP-850+ (undistilled product blend)	33	12 (10)
BaP (25 µg)	86	125 (100)
Solvent control	13	4 (3)
<u>ITSL^g</u>		
<650	50	31
IBP-850+ (TLP)	88	78
Solvent control	14	7

^aPercentage of mice with tumors in a group of 30 animals

^bTotal number of tumors normalized to a group of 30 animals; values in parentheses are normalized as a percent of BaP response

^c1:1 dilution of coal liquid with acetone; 50 µl initiating dose applied $\cong$ 25 mg

^d850°F+ tested separately from other bp cuts; all tested at 25 mg equivalent dose

^e1:1 dilution of coal liquid with acetone; 50 µl initiating dose applied $\cong$ 25 mg

^fDiluted at 0.55/ml and 50 µl dose applied

^gNon-laboratory-distilled materials, i.e., process-distilled material from pilot plant origins; 1:1 dilution of coal liquid with acetone or MeCl₂; 50 µl initiation dose applied $\cong$; different experiments

initiating activity for its bp range; its activity was, however, markedly lower than the full-boiling-range TLP (see Table 21). The initiating activity expressed in the ITSL sample may be due to the presence of carryover of higher-boiling materials in the <650°F cut during the crude process distillation. All other coal liquefaction materials studied were laboratory-distilled, after production.

In an effort to determine the chemical classes of the distillate fractions that were responsible for the tumor-initiating activity of these materials, alumina separations were performed and the fractions tested in the I/P assay. Figures 13 and 14 show that, for the two highest boiling SRC-II distillates, tumorigenic activity resided in the neutral PAH fraction. In both cases, the neutral PAH fractions were more active than their corresponding crude parent materials. A lower level of initiating activity was also detected in the NPAC fractions of the 800-850°F and 850°F+ bp cuts. Therefore, from these data, it appears that the neutral PAH constituents of these high-boiling SRC-II distillate fractions are the principal initiators of dermal tumorigenicity. More recent results have indicated that for all SRC-II distillates boiling above 700°F, the neutral PAH fraction is the most active in the I/P tumorigenicity assay.

Further subfractionation of the neutral PAH fraction of the two highest boiling SRC-II bp cuts was achieved by reverse-phase HPLC (See Methods). Four subfractions for the 850°F+ and eight subfractions for the 800-850°F cut were collected and tested in the I/P assay in an effort to isolate mass-specific fractions and determine the active tumor-initiating species of PAH in these class fractions. HPLC Subfraction 1 was the most active for the SRC-II 850°F+ bp cut (see Figure 15). The activity of this subfraction was comparable to the unfractionated PAH isolate. The other three HPLC subfractions expressed lower but significant activities, particularly Cuts 2 and 4. A detailed listing of the major components in HPLC Cut 1 was provided in Table 10, in the chemistry discussion section. The major PAH compound in this fraction was BaP. Table 22 provides both chemical and tumorigenicity data for the HPLC subfractions from the SRC-II 800-850°F PAH fraction. Subfractions that contained parent, C₁-, and C₂-substituted PAH were the most active tumor initia-

FIGURE 13. Mouse-Skin Tumor Response after Initiation with Chemical Class Fractions Prepared from the SRC-II 800-850°F Distillate Cut. A. Percent tumor incidence versus time. B. Cumulative skin tumor yield (normalized to 30 mice/group) versus time. A₁ = Aliphatic hydrocarbons; A₂ = Neutral PAH; A₃ = NPAC; A₄ = Hydroxy-PAH. Fractions were applied according to their relative weight distribution in the crude; 17 mg of the bp cut used.

FIGURE 14. Mouse-Skin Tumor Response after Initiation with Chemical Class Fractions Prepared from the SRC-II 850°F+ Distillate Cut (see Figure 13 for Fraction Nomenclature). A. Percent tumor incidence versus time. B. Cumulative skin tumor yield (normalized to 30 mice/group) versus time.

FIGURE 15. Tumor Yield after Initiation with HPLC Fractions of the Neutral PAH Fraction Isolated from the SRC-II 850°F+ bp Cut

TABLE 22. I/P Tumorigenicity and Chemistry Results for the HPLC Subfractions of the SRC-II 800-850°F Neutral PAH Fraction

Fraction	% Tumor Incidence	Adjusted Number of Tumors ^a	Wt% Distribution	Most Abundant Masses (m/z)	Mass Range (m/z)	Most Abundant PAH Compounds in Fraction	Concentration (ppt)
Crude PAH Fraction	63 88	34 88	-- 49	-- 242,215/216, 256,252	---	---	35.2 ± 5
PAH Subfractions- HPLC #							
1	57	39	2.9	228,234	226-229	Chrysene and/or triphenylene Benz[a]anthracene	245 ± 51 82 ± 13
2	90	76	7.2	242,252	240-252	C ₁ -benz[a]anthracene Benzo[j or b] fluoranthene 3-Methylchrysene	158 ± 2 91 ± 12 61 ± 1
3	90	84	3.9	254,242,252	242-254	Binaphthyls C ₁ -benz[a]anthracene	177 ± 37 128 ± 30
4	85	73	7.1	242	226-242	6- and/or 4-Methylchrysene	380 ± 47
5	80	59	19.0	256,244,268	244-268	C ₂ -Benz[a]anthracene and or C ₂ -chrysenes	128 ± 21
6	50	30	23.7	258,270,272	258-284	C ₃ -Benzofluorene and/or C ₄ -pyrene/fluoranthene	21 ± 2
7	23	12	14.4	288,274,298, 310	272-310	C ₄ -Benzofluorene and/or C ₅ -pyrene/fluoranthene	27 ± 2
8	64	21	22.0	288,300,302	276-328	C ₅ -Benzofluorene and/or C ₆ -pyrene/fluoranthene	6 ± 1

^aNormalized to 30 mice per group

tors. Conversely, subfractions with higher numbers of alkyl sidechains, $>C_3$, were much less active.

Based on the I/P results from SRC-II materials after alumina class fractionation and HPLC separation, it would be expected that the PAH fractions would be the most active tumor initiators for the other coal liquefaction materials considered in this report. Lower but significant activity may be expected in their NPAC fractions. Furthermore, activity in the higher-boiling PAH fractions would be expected to be due to parent, C_1 , and C_2 PAH. Materials with relatively high levels of alkylated PAH, such as the EDS process distillate cuts, would be expected to demonstrate lower tumor-initiating activities. This was shown to be the case when comparing the SRC-II and EDS distillate fractions (Later et al., 1984).

CHO Cell Mutagenicity

Mutagenic responses in the CHO cell assay of distillate cuts from wide-boiling-range SRC-I and -II coal liquids and two ITSL distillate fractions are summarized in Figure 16. The most mutagenically active coal liquid tested in the CHO assay was the SRC-II 850°F+ distillate cut, which was about eight times less active than BaP. The highest boiling distillate cuts of both the SRC-I and -II coal liquids were more active than their lower boiling counterparts. Comparing processes, the most active was SRC-II, followed by SRC-I, then by the ITSL materials (Frazier and Samuel, 1985).

Mutagenic responses of the chemical class fractions isolated from the SRC-II 850°F+ distillate materials are given in Table 23. All four fractions were active in the CHO assay. The PAH fraction was at least twice as mutagenic as the others (Mahlum et al., 1983).

Ecotoxicity (Special Blends)

The objective of the ecological toxicity screening studies was to aid in identification of engineering strategies that may be used to reduce concentration of toxic WSF components in coal liquefaction materials. These studies, in conjunction with characterization of bioactive agents in parent materials,

FIGURE 16. Mutagenic Potency of Distillate Cut Materials from Three Coal Liquefaction Processes in the CHO Assay

TABLE 23. CHO Cell Mutagenicity of Chemical Class Fractions Isolated from SRC-II 850°F+ Distillate Material

Chemical Class Fraction	Mutants/μg	Range of Activity (μg/ml)	Correlation Coefficient
AH	0.9	1-10	0.9
PAH	4	1-25	0.82
NPAC	1.8	1-15	0.7
Hydroxy-PAH	1.7	1-10	0.73
Crude	1.4	1-20	0.9

provide information useful in determination of overall environmental hazard of coal-derived materials. Research focused on characterizing WSF constituents from specially reblended groups of bp cuts from SRC-II, EDS and H-Coal materials. Differences in solubility of the bp cuts and chemical composition of their WSF were related to acute toxicity observed for the freshwater invertebrate, D. magna.

Acute Toxicity of WSF. Results of toxicity tests with D. magna are summarized in Table 24. Although the bp cuts from each material differed with respect to concentration and composition of their WSF, trends in toxicity were evident. For example, WSF from the higher bp cuts (700°F+) were always much less toxic than the lower and middle temperature range cuts based on percent solution. That is, a greater amount of the WSF was needed to elicit an acutely toxic response. This can be explained, in part, by the greater aqueous solubility of the lower bp cuts. WSF derived from 700°F+ cuts ranged from only 2 to 11 mg/L TC, while WSF from the <400°F cuts ranged from 730 to 1975 mg/L TC.

Differences in relative toxicity among bp cuts were not as evident when acute toxicity was expressed as concentrations of TC in solution (Table 24). In all cases, WSF from the 700°F+ bp cuts appeared to be more toxic when the 48-hour LC₅₀ was evaluated based on concentration of TC. Thus, although the higher boiling cuts were less soluble and may pose less of a hazard (on a vol-

TABLE 24. Acute Toxicities of WSF Derived from the Distillate Cuts to D. magna

Material	bp Range (°F)	WSF TC (mg/L)	48-Hour LC ₅₀ ^a	
			% WSF	TC (mg/L)
SRC-II	300-850+	300	0.5 - 1.1	1.5 - 3.4
	300-700	417	0.6 - 1.0	2.2 - 4.1
	700-850+	2	44.0 - 58.5	0.9 - 1.2
EDS	150-400	730	1.2 ± 0.8 ^b	8.9 ± 5.9 ^b
	400-700	220	2.6 - 3.7	5.6 - 8.2
	700-800+	5	41.2 - 53.0	1.9 - 2.4
H-Coal	<400	1975	0.1 - 0.2	4.3 - 14.9
	400-700	445	0.6 - 1.0	2.9 - 4.4
	700-850+	11	11.0 - 13.0	1.2 - 1.5

^aValues given as the range of two measurements

^bThree determinations; mean ± standard deviation (SD)

ume basis), if spilled or released to aquatic environments, their soluble constituents exhibited greater toxicity than constituents derived from the lower boiling range cuts.

Overall, the acutely toxic range of the materials tested (0.9 to 14.9 mg/L TC) approximated that of other coal liquids with similar characteristics (Dauble et al., 1983; Later, 1985). It would be inappropriate to make comparisons among materials because of differences in the bp intervals tested and the small sample size. Additionally, previous studies have shown that process conditions can have considerable effect on parent material composition and resultant toxicity (Dauble et al., 1983; Later, 1985).

Chemical Characterization of WSF. Although previous studies had shown that phenols usually comprised more than 90% of the soluble constituents in full bp range coal liquids, it was not known to what extent the hydrocarbon and nitrogen classes would contribute to the chemistry and toxicity of the WSF

of the bp cuts. Therefore, for the present studies, the concentrations of these three major chemical classes in WSF from two of the materials tested were compared.

Detailed chemical analyses of the water-soluble phenols indicated that concentrations of total phenols declined as the bp range increased (Table 25). WSF for bp cuts <400°F had total phenol levels that were elevated three to five times above those of the 400-700°F cuts. Boiling point cuts >700°F contained negligible amounts of phenols. Differences among the phenol composition of the bp cuts were also evident. For example, cresols and C₂-phenols collectively comprised more than 99% of the total phenols in the >400°F cuts but only about 40% of the total in the 400-700°F range, where the C₂ and C₃ phenols predominated.

Total saturated and total aromatic hydrocarbon concentrations in WSF from the EDS material increased with decreasing bp range (Table 26). In contrast, WSF derived from the 400-700°F cuts of H-Coal material contained the highest concentrations of both aromatic and saturated hydrocarbons. The C₄-benzenes were the major aromatic hydrocarbon constituents of the <400°F cut WSF and comprised 43 and 90% of the totals for EDS and H-Coal materials, respectively. WSF from the 400-700°F bp cuts were enriched in naphthalene and substituted naphthalenes.

These studies provided the first data on relative amounts of total nitrogen bases in WSF from coal liquefaction materials other than SRC. Analysis indicated that this class is second to phenols in terms of concentration in the WSF (Table 27). Concentrations of total nitrogen bases were elevated two to five times above those measured for the total aromatic hydrocarbon fraction within a comparable bp range. Highest concentrations of nitrogen bases were found in the 400-700°F range cuts from the H-Coal material: levels approximately 14% of those noted for total phenols. Anilines comprised the majority of the NPAC bases in WSF derived from EDS cuts >700°F. Increased ring substitution was noted in the higher bp cuts from both EDS and H-Coal.

TABLE 25. Phenol Concentrations in WSF from the SRC-II, EDS and H-Coal Re-blended Distillate Cuts. Concentrations (mg/L) expressed as means of duplicate samples

Compound	Boiling Point Range (°F)		
	<u>SRC-II</u>		
	<u>300-700</u>	<u>300-850</u>	<u>700-850+</u>
Phenol	62.2	49.6	<0.01
Cresols	97.2	69.9	<0.01
C ₂ -phenols	62.9	42.2	<0.01
C ₃ -phenols	36.2	25.1	<0.01
C ₄ -phenols	18.8	13.0	<0.01
>C ₅ -phenols	15.4	12.1	<0.01
Indanol	13.4	8.6	<0.01
C ₁ -indanols	8.6	5.4	<0.01
C ₂ -indanols	2.4	5.4	<0.01
Total phenols	317.2	227.9	<0.01
 <u>EDS</u>			
	<u>150-400</u>	<u>400-700</u>	<u>700-800+</u>
Phenol	88.6	0.3	<0.01
Cresols	313.5	2.3	<0.01
C ₂ -phenols	136.6	61.9	<0.01
C ₃ -phenols	4.3	64.7	<0.01
C ₄ -phenols	<0.01	15.3	<0.01
>C ₅ -phenols	<0.01	10.2	<0.01
Indanol	<0.01	10.7	<0.01
C ₁ -indanols	<0.01	3.7	<0.01
C ₂ -indanols	<0.01	1.2	<0.01
Total phenols	543.0	170.4	0.04
 <u>H-Coal</u>			
	<u><400</u>	<u>400-700</u>	<u>700-850+</u>
Phenol	60.6	0.3	<0.01
Cresols	1261.9	4.1	<0.01
C ₂ -phenols	550.5	138.3	<0.01
C ₃ -phenols	<0.01	116.6	<0.01
C ₄ -phenols	<0.01	17.6	<0.01
>C ₅ -phenols	<0.01	15.7	<0.01
Indanol	<0.01	49.4	<0.01
C ₁ -indanols	<0.01	9.5	<0.01
C ₂ -indanols	<0.01	2.3	<0.01
Total phenols	1873.0	353.6	3.0

TABLE 26. Concentration of Hydrocarbons in WSF Fractions from the EDS and H-Coal Distillate Cuts. Values given as mean of duplicate samples

Material	bp Range (°F)	Total Aromatic Hydrocarbons (µg/L)	Total Saturated Hydrocarbons (µg/L)
EDS	150-400	45	8995
	400-700	21	3099
	700-800+	2	57
H-Coal	<400	8	8014
	400-700	273	9957
	700-850+	4	15

TABLE 27. Concentration of Nitrogen Bases in WSF from the EDS and H-Coal Distillate Cuts. Values given as mean of duplicate samples

Material	bp Range (°F)	Total Nitrogen Bases (µg/L)
EDS	150-400	16600
	400-700	11200
	700-800+	11
H-Coal	<400	301000
	400-700	40500
	700-850+	52

CONCLUSIONS

Results of the biological assays of the 50°F distillate cuts of coal liquefaction materials from the SRC, H-Coal, EDS and ITSL processes have been in general agreement: materials boiling above 700°F show significant biological activity, with that activity generally continuing to increase with increasing bp. Regardless of process, it appears, therefore, that limiting the upper distillation cut temperature of the material is an effective strategy to reduce the potential health hazards associated with exposure to coal liquefaction products. Recycling the heavy ends to extinction, for example, significantly decreased the biological activity of certain SRC-II process materials (Wilson et al., 1983b). Limiting the product distillate from the ITSL process to an upper cut point temperature of 650°F effectively eliminated mutagenic activity in this material as well (Wilson et al., 1983a).

Results from chemical analyses of 50°F distillate cuts of coal liquefaction materials from the SRC, H-Coal, EDS and ITSL processes have shown some differences in the final products. These appear to be a function of the processes rather than of the coal type. In general, those materials from processes that incorporate some form of catalytic hydrogenation (i.e., the EDS and ITSL) have higher concentrations of alkylated and hydrogenated species, with lower concentrations of amino-PAH and azaarenes. Mutagenic activity was generally reduced in the hydrotreated liquids as compared to the single-stage products. However, there was no significant difference in the initiating activity between materials of the two types of processes for a given bp cut temperature range.

Fillo and Craun (1985) have suggested that the 95% over distillate cut point be used as a single number parameter to predict the toxicologic activity of coal-derived materials that have not undergone postproduction hydrotreatment. The 95% over distillate cut point is defined as the temperature at which 95% of the product has been distilled. When all available data were considered for the processes of concern in this report, it was shown that those materials with 95% distillate cut points above 640°F were active with a statistical significance of $P \leq 0.05$.

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