Chemical, Biomedical and Ecological Studies of SRC-I Materials from the Fort Lewis Pilot Plant: A Status Report

D. D. Mahlum

January 1981

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CHEMICAL, BIOMEDICAL AND ECOLOGICAL STUDIES
OF SRC-I MATERIALS FROM THE FORT LEWIS
PILOT PLANT: A STATUS REPORT

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FOREWORD

This report presents the results of initial chemical, biomedical and ecological studies with a number of solvent refined coal (SRC-I) materials. These materials were supplied by the Pittsburgh & Midway Coal Mining Co. from the U.S. Department of Energy pilot plant at Fort Lewis, Washington. These materials have been used to develop methodology which can be used for a more refined analysis of materials relevant to a commercial SRC-I technology. Although much detail has been omitted in order to avoid producing an unduly long document, sufficient technical data have been included to provide a perspective of the efforts to date.

EXECUTIVE SUMMARY

This document discusses studies performed with solvent refined coal (SRC) materials obtained from the Fort Lewis Pilot Plant during operation in the SRC-I mode. The development of analytical methodology is presented as well as results obtained from the application of these methods to light oil (LO), wash solvent (WS) and process solvent (PS). Results of cellular and animal studies with LO, WS and PS are included, along with a description of methods developed for the generation and characterization of LO and PS aerosols, and for exposing rats, mice and guinea pigs to these aerosols. The effects of SRC-I product on seed germination and plant growth which have also been studied are discussed.

The SRC-I product, feed coal and the mineral residue have been analyzed for inorganic constituents using neutron activation analysis. These data indicate greatly decreased concentrations of most mineral elements in the SRC-I product relative to the feed coal. Chromium, nickel, lutetium, tantalum, and zinc all showed less than a 5-fold reduction in concentration in product relative to coal; most other elements showed a 10- to 80-fold decrease. Bromine, titanium and mercury were exceptions, appearing in nearly equal concentrations in coal and SRC-I product.

Organic constituents of SRC materials have been characterized using solvent fractionation, gas chromatography (GC), gas chromatographic mass spectrometry (GCMS), high-resolution mass spectrometry (HRMS), LH-20 Sephadex chromatography and high-pressure liquid chromatography (HPLC). The light oil consisted of about 80% aliphatic, 10% aromatic, and 5% acidic compounds; wash solvent contained about 50%, 12% and 27% of these components, respectively, when fractionation was performed by solvent extraction. Process solvent was composed of a larger aromatic fraction than LO and WS and a substantial "tar" fraction which is insoluble in isooctane. Using GC, GCMS and HRMS, a wide variety of compounds have been identified in LO, WS and PS. Among those which may be of potential biomedical or ecological concern are phenols, polynuclear aromatics, and nitrogen bases, including primary aromatic amines.

The higher-boiling-point material, PS (b.p. range, 450° to 850°F), exhibited significant mutagenic activity in the Ames assay; LO and WS were inactive. Process solvent also caused transformation of cultured Syrian hamster embryo cells. Light oil and WS were not active in this assay. The basic fraction (derived by solvent fractionation) from PS was enriched in mutagenic and transforming activity (activity/μg) compared to the unfractionated material. Additional chemical fractionation studies suggest that primary aromatic amines are major determinants of the observed mutagenic activity.

Although none of the SRC-I materials has been subjected to skin-painting assay for carcinogenesis, skin-painting studies with SRC-II naphtha, heavy distillate, shale oil and petroleum crude indicate a good correlation between the results of the cellular assays and skin carcinogenesis in mice.

Wash solvent was more toxic after oral administration to rats than was light oil or process solvent (acute LD₅₀ = 0.56 g/kg for WS, 2.8 to 2.9 g/kg for light oil and process solvent). When the materials were administered once daily for 5 consecutive days, indications of cumulative toxicity were found for PS. Cumulative effects, if present, were slight for LO and WS.

The effects of LO, WS and PS on development were studied after administration to pregnant rats from 7 to 11 or 12 to 16 days of gestation (dg). Fetal growth and survival were decreased by all materials at either period at doses approaching maternal toxicity. Similar doses of process solvent also increased the incidence of fetal abnormalities (primarily cleft palate) after administration from 12 to 16 dg.

The tissue distribution of a number of components of PS was studied after oral administration of PS to rats. The rate of loss of the measured compounds from the stomach was found to be inversely proportional to the compound molecular weight. Detectable amounts of the selected compounds in blood were found only in red blood cells at the times studied. Liver and fat generally contained the highest concentrations of the compounds analyzed.

The effect of SRC-I product on the germination and growth of barley was investigated by mixing or layering the product with soil and placing the mixture in a field lysimeter. When soil and product were mixed 1:1, little effect on germination, growth or yield was found. When the barley was grown on a 3-dm layer of soil placed over a 1-dm layer of product, growth and yield were reduced, apparently due to inability of roots to penetrate the product layer.

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CHEMICAL, BIOMEDICAL AND ECOLOGICAL STUDIES OF SRC-I MATERIALS
FROM THE FORT LEWIS PILOT PLANT: A STATUS REPORT

1.0 INTRODUCTION

1.1 OVERVIEW: DOE'S SRC ENVIRONMENTAL PROGRAM

In parallel with the process development for the commercialization of economically viable coal conversion technologies, DOE has established a program of investigation, assessment and control to ensure the environmental acceptability of these processes. DOE's environmental program has been outlined generically for liquefaction processes in the Environmental Development Plan (DOE/EDP-0012, March 1978) and has been addressed programmatically for SRC-I and SRC-II in a Project Environmental Plan (PEP). The environmental program presented in the PEP is implemented jointly by Fossil Energy (FE) and the Office of Environment (EV).

1.2 PNL'S ROLE IN THE ENVIRONMENTAL EVALUATION OF SRC-II

A number of organizations have been selected by DOE to evaluate the biomedical, environmental, and safety aspects of SRC-I. The International Coal Refining Company has been assigned responsibility for industrial hygiene. Oak Ridge Operations has been assigned responsibility for the writing of necessary Environmental Impact Statements (EIS's) and the filing for EPA construction permits. In this context Pacific Northwest Laboratory, (PNL) has been assigned responsibility for: 1) generation of a biomedical and ecological data base for materials from the SRC processes, and 2) the development and application of appropriate protocols for the interpretation and use of these data for assessment of health and environmental impacts for the SRC processes.

1.3 STATUS OF THE PNL PROGRAM

Pacific Northwest Laboratory initiated biomedical and environmental studies with SRC materials in FY 1978 as part of an overall synthetic fuel research effort. As an outgrowth of this research, PNL was requested to

develop a comprehensive Program Plan for the evaluation of the health and ecological aspects of the SRC processes. A preliminary Program Plan relevant to the SRC-II process was prepared in the first quarter of 1979 for review by ET, EV, and P&M staff. A detailed environmental plan for SRC-I is being drafted at this time and should be available early in 1981. A summary of this plan has been submitted to DOE.

The present status report constitutes a technical presentation of the results of the PNL research efforts which form much of the basis for the detailed environmental plan. As such, it is one of a series of regular status reports by PNL on coal liquefaction program activities, findings, and conclusions.

1.4 OUTLINE OF PRESENT STATUS REPORT

Pacific Northwest Laboratory recognizes the critical role of representative sampling for health and ecological characterization. These samples should be chosen in a manner allowing extrapolation of the data obtained from pilot-plant, PDU, and bench-level materials to demonstration and commercial SRC materials. This concept defines "scalable" for the purposes of this document. Accordingly, this and future reports will address two complementary issues: a) provenance of samples used in relation to pilot-plant operations; and b) the relationship of pilot-plant samples to potential demonstrational/commercial operations. This is followed by a comprehensive synopsis of PNL's experience and findings to date regarding the chemical, biological, and ecological characteristics of SRC materials with particular emphasis on SRC-I materials.

2.0 SAMPLING

2.1 SRC-I TECHNOLOGY: NEWMAN, KY, SRC-I DEMONSTRATION PLANT

The SRC-I process is a coal-conversion process in which coal is hydrogenated to produce a variety of solid, liquid, and gaseous products. These products can be utilized directly or further upgraded before use. Although the process chemistry is similar to that of other direct liquefaction processes, the differences in the details of these processes can perhaps lead to somewhat different environmental and toxicological impacts. Furthermore, the SRC-I Demonstration Plant design differs from the pilot plants and PDU's at which the process has been investigated. For this reason it is worthwhile to compare these cases separately before discussing the conditions under which samples were obtained for this study.

Details of the engineering design are being prepared as part of Phase I of the U.S. Department of Energy SRC-I demonstration project. The basic process unit operations have been selected, although some unit operations are still being evaluated as Phase I engineering design continues, especially in some areas related to environmental and emissions control. The design of the primary processes is, however, complete enough to allow comparison of the process which produced materials used in the present study to that of the Demonstration Plant.

The primary factors which are generally considered to control the nature of the products of the coal liquefaction reactions are: the feed coal, reaction conditions in the dissolver, and the nature of the recycle process solvent. Measurable reaction conditions of interest specifically include: coal concentration in the feed slurry, dissolver outlet temperature, dissolver total pressure, recycle hydrogen purity, dissolver space rate (i.e., residence time for the liquefaction reactions) and hydrogen-to-coal feed ratio. Also thought to be important, but difficult to measure, is the solids' concentration in the dissolver. The nature of the recycle solvent is extremely difficult to characterize quantitatively. Since it is a product of the reaction and is recycled, the most important factor (for any particular feed coal and set of operating

conditions) is probably whether the system has been operated long enough to eliminate start-up solvent and establish a generally steady-state composition of the recycle solvent.

It is clear that the nature of the chemical compounds produced depends on the feed coal as well as the conditions of the reactions. Because such a wide variety of reaction products is produced, the species distribution (and perhaps even the species types) will vary considerably if either the feed coal characteristics or reaction conditions change significantly. It is probable that many compounds will have the same generic nature, and in fact that many of the exact same compounds will be present as long as reaction conditions are close to their nominal value. However, percentage distribution of individual species is almost certain to vary, and the percentage distribution of generic compound types can be expected to vary as well.

The nature of the SRC materials produced will also depend on the separation methods used to recover light liquid products produced in the primary liquefaction reactions and to remove ash and unreacted carbon. These separation steps may alter the chemical composition of the SRC and the process solvent, and also the concentration of various chemical species.

The biological activity of complex mixtures of hydrocarbons such as coal liquefaction products is expected to depend strongly on the particular compounds present and their concentrations. Consequently, sampling to obtain products for testing needs to be done under conditions where the liquefaction reactions are as close as possible to the design basis for the Demonstration Plant. This will ensure that essentially the same compounds appear in the reaction products.

The samples used in this study were obtained from the Fort Lewis Pilot Plant in December 1976 (liquids) and March 1977 (solid products). The Fort Lewis Pilot Plant differs somewhat from the design of the Demonstration Plant. Some of these differences are the result of the requirements to operate in the SRC-II mode, but most are due to the scale difference and the different development goals of the Pilot Plant and Demonstration Plant. Several major process areas are not present at Fort Lewis such as coal and mineral ash residue

gasification, product workup by coking/calcing or hydrotreating, sulfur recovery by Claus process, and onsite solid waste disposal. Hydrogen sulfide recovery and recycle gas cleanup are accomplished by different processes than in the Demonstration Plant (DEA absorption of H_2S and naphtha absorption of hydrocarbons are used at Fort Lewis).

At the time the samples were taken, mineral ash residue was removed from the product at Fort Lewis by filtration, rather than supercritical solvent de-ashing, as is planned for the Demonstration Plant. The other differences most significant to sampling for this study are in the fractionation and vapor/liquid separation systems. The fractionation system at Fort Lewis was designed to match the requirements of a flexible pilot plant reaction system and to meet the process solvent and filtration wash solvent requirements of that plant. The fractionation system at the Demonstration Plant will be designed to produce liquid products suitable to the commercial market. Due to the necessary differences in tower design, the liquid product fractions will have different boiling point ranges. Even if the boiling point ranges were specified to be nominally the same, the specific chemical compositions would differ somewhat from those at Fort Lewis. The Fort Lewis fractionation system produces a process solvent with a nominal boiling point range equivalent to that currently planned for the Demonstration Plant. It also produces a wash solvent and a naphtha which are somewhat different from analogous streams slated for the Demonstration Plant.

2.2 SAMPLING FOR THIS STUDY

The samples used in this study were produced at Fort Lewis as part of the extended production run to supply materials for the SRC burn test. Liquid samples were taken directly from the process in December 1976 during the last stages of this run. The solids sample was obtained in March 1977 from product storage at Fort Lewis from materials produced in December under essentially the same process conditions as the liquid products.

Records are not complete for all operating parameters at the time these materials were produced. The pertinent operating conditions and materials

properties (for which these records are available) under which these samples were produced are summarized in Table 1, along with analogous conditions for the Demonstration Plant design basis.

The primary reaction conditions for the Fort Lewis samples are similar to, but not identical with, the design basis conditions for the Demonstration Plant. Feed coal was from the Kentucky No. 9 and 14 seams, which is the design coal. This feed coal is close to the nominal chemical analysis (and within the specified limits of variability) for the design basis. Both chemical analysis and petrographic compositions of coals can vary from mine to mine within a single seam. Given the normal variability of coals, these coals are considered to suitably represent feed for the Demonstration Plant.

De-ashing of the solid product was by filtration rather than supercritical solvent de-ashing as is planned for the Demonstration Plant. The solid product would be expected to contain the same types of chemical compounds as will be expected from the Demonstration Plant, but proportions of individual compounds may be expected to vary somewhat.

Samples were taken during the late stages of a production run. During this run, make-up process solvent was added at intervals to keep the process solvent inventory in balance. This coal liquid make-up solvent was not derived

TABLE 1. Operating Conditions at Time of Sampling for Preliminary Screening

<u>Condition</u>	<u>Fort Lewis (measured value)</u>	<u>Phase I Design Basis (calculated value)</u>
Feed coal (MF)	KY #9 and # 14	KY #9
% C	70.76%	71.0%
% H	5.12%	5.0%
% N	1.47%	1.5%
% S	3.96%	3.7%
% O	7.77%	8.4%
% Ash	10.43%	10.5%
Coal feed concentration	36.9% - 37.7%	38.5%
Dissolver temperature	850°F	840°F
Total pressure	1500 psig	2000 psig

from the SRC process but during a different run, and would thus have different hydrogen donor characteristics than steady-state process solvent from the Demonstration Plant. At any given time, however, a large part of the process solvent was probably SRC process derived. Because of this make-up solvent addition, as well as variations in normal pilot plant operation, the chemical composition of process solvent should vary somewhat from that of a solvent produced solely by long-term operation at the design conditions.

Three different types of liquids were obtained from Fort Lewis, as summarized in Table 2. It is felt that these product streams contain essentially the same chemical compounds as will be produced by the Demonstration Plant coal liquefaction reactions, and that the total amounts of the individual compounds produced will be similar. The SRC solid product samples are expected to be similar, but not identical to Demonstration Plant solid product. The liquid samples are thought to contain the same chemical compounds as the Demonstration Plant liquids, but distributions of species may differ (Table 3).

Because of different fractionating systems, it is expected that the various chemical species (perhaps including those with the greatest degrees of biological activity) will distribute differently between naphtha (light oil), wash solvent and process solvent stream than they would for the Demonstration Plant liquid products. The initial tests reported in this study will be used to provide guidance for later testing of materials produced at the Pilot Plant under conditions directly analogous to the Demonstration Plant design, as well as to provide early guidance in selection and design of the Demonstration Plant control measures.

3.0 CHEMICAL CHARACTERIZATION

3.1 INITIAL ORGANIC ANALYSES

Small samples of SRC-I materials were first obtained from the Fort Lewis Pilot Plant in 1975 for preliminary development of chemical characterization methodology. Initially, gas chromatography with packed glass columns using a flame ionization detector was used to separate and identify the organic compounds present in these materials. The complexity of the mixtures, as indicated by the large number of gas chromatographic peaks, made clear the need for a separation scheme which would yield simpler fractions. Since identification was based solely on comparison of retention-time data with known standards, fractions having fewer components were necessary.

A solvent-solvent separation technique based on polarity differences of organic compounds was chosen (Figure 1). The organic solvent chosen was isooctane, since published procedures showed that use of this solvent allowed separation of aromatic from aliphatic compounds when dimethylformamide was used as a counter-solvent. We found evidence, however, that dimethylsulfoxide (DMSO) was superior to dimethylformamide as a counter-solvent and the separation technique finally evolved into the following scheme depicted in Figure 1.

With process solvent (PS) in addition to the standard acidic, basic, aliphatic and aromatic fractions, insoluble materials separated out after addition of isooctane, NaOH and HCl. These insoluble materials have been designated as neutral, acid and basic "tar fractions." Similar tar fractions have been obtained from solvent fractionation of SRC-II heavy distillate.

The small quantities of the SRC-I samples used for these initial studies were obtained before the filtration unit was in service at the Fort Lewis Pilot Plant. Therefore, no wash solvent cut was being produced and the SRC-I product contained all of the ash and unreacted coal. A set of samples, including L0, WS, PS and mineral residue, obtained in substantial quantities from the Fort Lewis Pilot Plant in December 1976 were used as research materials for most of this research. An additional sample of SRC-I product, obtained in March 1977,

FIGURE 1. Solvent Fractionation of SRC Materials

was produced under the process conditions described in Section 2.0 on "SAMPLING." Some chemical and physical properties of the research materials are presented in Table 2.

The L0, WS and PS were fractionated using solvent extraction. The results are shown in Table 3. The recoveries are related to their boiling ranges.^(a) Since each fraction was concentrated by removing the isooctane solvent, recovery improved as the boiling range shifted to higher temperatures. The relative proportions of the light-oil fractions may be misleading because of the loss of volatile compounds reflected in the low recovery. The loss of volatiles is a disadvantage of separation techniques in which a concentration step is used.

Gas chromatographic analysis of the fractions obtained by solvent extraction used methyl silicone liquid phases on deactivated Chromosorb W support with flame ionization. Compounds in the fractions were identified by comparing retention times of chromatographic peaks from the fractions with those of authentic standards. Table 4 shows some of the classes of compounds identified in fractions of PS by this method.

(a) For L0, ambient to 380°F; WS 380 to 480°F; PS 480 to 850°F.

TABLE 2. Physical and Chemical Characteristics of SRC-I Research Materials

Material	Density ^(c)	Boiling Range (°F) ^(d)	Percentage ^(c)					Other
			C	H	N	S	O ^(f)	
Light oil ^(a)	0.725	amb to 380	84.32	12.98	0.21	1.96	0.53	
Wash solvent ^(a)	0.959	380 to 480	82.84	9.03	0.39	0.47	7.27	
Process solvent ^(a)	1.043	480 to 850	88.63	8.05	0.81	0.63	1.88	
SRC-I product ^(b)	--	330 (fusion point ^(e))	86.71	5.89	2.06	0.76	4.46	0.13% ash

(a) Obtained by PNL personnel.

(b) Shipped by P&M personnel.

(c) Provided by Physical Sciences Department, PNL.

(d) Nominal ranges only.

(e) Determined by P&M.

(f) Oxygen content determined by difference.

TABLE 3. Composition (Approximate %) of SRC-I Oils

<u>Fraction</u>	<u>Light Oil</u>	<u>Wash Solvent</u>	<u>Process Solvent</u>
Aliphatic	80	53	32
Aromatic	10	12	29
Acidic	5	27	10
Basic	5	8	3
Insolubles	0	0	26
NET RECOVERY OF ORIGINAL MATERIAL	30%	90%	110%

TABLE 4. Compounds Found in Fractions of Process Solvent

<u>Fraction</u>	<u>Compounds</u>
Aliphatic	N-alkanes, cyclohexane, decalin
Polynuclear aromatic	Naphthalene, methylnaphthalenes, tetralin, biphenyl, phenanthrene, pyrene, fluoranthene
Acidic	Phenol, cresols, xylenols, trimethylphenol
Basic	Quinoline, methylquinolines, carbazole
Insoluble tar	Not determined

Gas chromatographic mass spectral analysis allowed confirmation of the identities assigned to the GCMS's various components on the basis of GC. It also became apparent that the early chromatograms contained many unresolved components. However, with improved chromatography and GCMS analysis, it was possible to resolve and identify all of the major peaks in the SRC-I liquids.

3.2 QUANTITATIVE CAPILLARY GAS CHROMATOGRAPHY

Methods were needed to measure the biological disposition (absorption and distribution) of several components in PS. A quantitative capillary GC method

was developed which allowed direct analysis of PS components without prior sample preparation: A 60-m SE-54 wall coated glass capillary column was purchased from J & W Scientific (Supelco) with greater than 160,000 effective plates at a k' of 5. The column was operated in split mode with a split of approximately 1:50 and a head pressure of about 38 psi helium. The injected volume was 2 μ l. The splitter body used a 2-mm ID glass liner packed with silanized glass wool. The injection port temperature was 350°C, detector temperature was 300°C, initial oven temperature was 100°C, final oven temperature was 220°C, and the program rate was 2.5°C/min. The initial oven temperature was held for 6 min before the temperature program started; the final temperature was held until the last peak of interest was eluted. Peak areas were determined by electronic integration, with the base line reset between all peak valleys.

Mass spectra were obtained on a Hewlett-Packard 5985 combined GCMS. A 60-m fused silica capillary column coated with SE-54 (purchased from J & W Scientific) was inserted through the GCMS interface line directly into the mass spectrometer ion source. The instrument was calibrated with perfluorotributylamine. The electron impact spectra were recorded at an ionization potential of 70 electron volts. The mass range of interest was scanned at 200 amu/sec. Data were stored on the computer for later retrieval. Mass spectra were interpreted and identified using reference spectra in the NBS spectral library and previously recorded data from several other sources.

A typical capillary chromatogram of SRC-I process solvent is shown in Figure 2. At least 100 compounds have been tentatively identified by GCMS. Of these, nine (numbered peaks on Figure 2) were selected for quantitation in preparation for biological studies. The retention times of these compounds in PS were then carefully compared with those of authentic standards.

Table 5 shows the reproducibility of the retention times for each compound for 10 runs of standards. Since the standards were not all of the same concentration, the retention times generally show a slight increase in the more concentrated samples. This effect results in retention-time standard deviations which are slightly larger than if the various compound concentrations had been equal for all runs. The variability of a C₂₀ internal standard (in these same runs, at the same concentration) showed a standard deviation of only 0.05 min with a retention time of approximately 55 min.

FIGURE 2. Capillary Chromatogram of Process Solvent. See Table 5 for identification of numbered peaks.

TABLE 5. Summary of Study on Reproducibility of Retention Times on Capillary Column Chromatography for Several Standards

Compound	Mean Time (min)	Standard Deviation	Coefficient of Variation
1. Naphthalene	16.01	0.04	0.20
2. 2-Methylnaphthalene	21.62	0.04	0.20
3. 1-Methylnaphthalene	22.55	0.04	0.19
4. Biphenyl	26.10	0.04	0.16
5. Acenaphthene	31.75	0.05	0.17
6. Fluorene	36.80	0.05	0.15
7. Phenanthrene	46.95	0.10	0.20
8. Fluoranthene	59.26	0.12	0.20
9. Pyrene	62.16	0.10	0.15

To quantitate selected PNAs, standard curves were prepared by chromatographing varying concentrations of authentic PNAs with decane as an internal standard. Plots of ratios of the peak areas to the peak area of decane were made for each PNA of interest. A typical plot of the data for naphthalene is shown in Figure 3. The data for each compound were fitted to a least-squares model and the values of r^2 , intercept and slope for each compound calculated. The results for all compounds are linear, with no r^2 value being less than 0.9995. The lines also show a small intercept. A force fit of the data through the origin results in a very small change in the slope for most compounds.

Concentrations of the nine PNA compounds quantitated are shown in Table 6. Phenanthrene and 2-methylnaphthalene are the two most abundant PNA compounds of the nine. Reproducibility of replicate injections for the same sample is also shown in this table. Most of the values replicate well, considering the complexity of the sample. In the few instances when the results using the integrated areas don't agree (naphthalene and fluoranthene), quantitation using peak heights produces results which agree within 0.3% for the two runs.

FIGURE 3. Standard Curve for the Quantitative Analysis of Naphthalene Using Capillary Column Gas Chromatography

TABLE 6. Reproducibility of Analysis of Selected Components of SRC-I Process Solvent

Compound	ppm		Percent Variation	
	Run A	Run B	Area	Peak Height
Naphthalene	7,305	7,649	4.7	0.3
2-Methylnaphthalene	34,881	35,008	1.1	--
1-Methylnaphthalene	5,519	5,489	0.1	--
Biphenyl	9,216	9,046	1.8	--
Acenaphthene	9,029	9,216	2.1	--
Fluorene	12,689	11,935	5.9	--
Phenanthrene	40,683	40,386	0.7	--
Fluoranthrene	5,226	6,209	18.3	0.3
Pyrene	4,854	4,820	0.7	--

The reliability of the method was further checked by spiking PS samples at two concentration levels with mixtures of the PNA compounds of interest. The results are shown in Table 7. The recoveries of all compounds studied ranged between 95.5 and 103.2%.

Summing the values for the compounds quantitated accounts for approximately 12% of the total SRC-I material injected. If all the integrated area from the GC run is used to calculate a total amount, the percent increases to about 30%, assuming the response for all peaks to be that of phenanthrene. A shift in the baseline indicates failure to resolve some materials, as well as the possible presence of higher-molecular-weight compounds. Approximating this area, and again assuming the response to be equal to phenanthrene, we can account for about 80% of the SRC-I material injected. These estimates are probably low for at least two reasons: 1) high-molecular-weight materials are lost in the injection port or do not elute from the column by the end of the run. 2) The run time was held to approximately 1 h because our primary interest was to quantitate the more concentrated compounds, most of which elute in the first 30 to 40 min.

TABLE 7. Recovery of Selected Compounds After Addition to SRC-I Process Solvent

Compound	ppm in PS	ppm Added	ppm Spike & SRC	Percent Recovery	ppm Added	ppm Spike & SRC	Percent Recovery
Naphthalene	7,649	1,353	9,018	100.2	13,373	20,791	98.9
2-Methylnaphthalene	35,008	893	36,004	100.3	8,834	43,861	100.0
1-Methylnaphthalene	5,489	589	6,068	99.8	5,808	11,030	97.6
Biphenyl	9,046	399	9,550	99.3	3,952	13,110	99.6
Acenaphthene	9,216	986	10,074	100.6	9,745	17,673	95.6
Fluorene	11,935	899	13,054	101.7	8,879	20,790	99.9
Phenanthrene	40,386	3,825	44,232	99.4	37,783	76,665	97.7
Fluoranthene	6,209	2,002	8,487	103.2	19,769	25,818	99.4
Pyrene	4,820	774	5,694	101.8	7,688	11,973	95.5

Results of this work indicate that the major compounds in SRC liquids can be analyzed quantitatively using glass capillary-column chromatography. This technique can be used to analyze major PNA compounds without prior separation by liquid-column chromatography. The methods and results presented here will be used in support of studies on the metabolism and pharmacokinetics of SRC liquids.

3.3 ADDITIONAL FRACTIONATION AND ANALYTICAL METHODS FOR ORGANIC CHARACTERIZATION

As chemical and biological characterization of SRC-I material proceeds, additional fractionation and analytical methods are needed. Several methods developed and applied to SRC-II liquids and to shale oil will be used for more detailed analysis of SRC-I materials. A description of these techniques and their areas of applicability is presented here. Data on SRC-I materials presented in this section are from samples which originated at the SRC-I pilot plant in Wilsonville, AL.

The solvent extraction method is useful for obtaining relatively large quantities of general classes of organic materials, but it is time-consuming, relatively harsh, and results in tar formation. Therefore, partition chromatography on Sephadex LH-20 and high-performance liquid chromatography are also being used to provide milder and more rapid separations, in quantities sufficient for cell culture and chemical characterization.

3.3.1 LH-20 Partition Chromatography

Sephadex LH-20 has been used in the partition mode by Klimisch and Stadler and their technique applied to fossil fuels by Jones et al. A modification of their technique has been used here in which the LH-20 is swollen with MeOH:H₂O (85:15) and the sample is eluted successively with hexane, 10% toluene in hexane, and methanol. This provides a polarity separation, the hexane fraction being the least polar and the methanol fraction the most polar. Table 8 indicates the types of compounds which may be expected to elute in each fraction, based on work with standards. The toluene/hexane fraction shows some overlap

TABLE 8. Standards on Sephadex LH-20

<u>Hexane Fraction</u>	<u>Toluene/Hexane Fraction</u>	<u>Methanol Fraction</u>
Aliphatic hydrocarbons	(Azaheterocyclics)	Carbazole
Polycyclic aromatic hydrocarbons	(Anthraquinone)	Indoles
Nitrofluorene		Primary aromatic amines
Dibenzofuran		Aza-aromatic amines
Dibenzothiophene		Diazaheterocyclics
Fluorenone		Naphthols
Cyanophenanthrene		Aminobenzothiadiazole

with both the hexane and methanol fraction, a fact which is useful in biological assays. Table 9 shows the weight percent distribution in these fractions for some materials tested.

3.3.2 High Performance Liquid Chromatography

A semipreparative, normal-phase NH_2 column was used with the ternary solvent gradient shown in Table 10 to analyze the LH-20 fractions described above, as well as the unfractionated material.

TABLE 9. Percent of Material Recovered from the Sephadex Method

	<u>Hexane</u>	<u>Toluene/Hexane</u>	<u>Methanol</u>	<u>Percent Recovery</u>
Paraho (shale oil)	82.6	2.4	4.7	89.7
Livermore (shale oil)	73.9	2.1	4.5	80.5
Vernal, Utah (shale oil)	81.7	2.0	3.6	87.3
Occidental (shale oil)	84.2	2.0	2.1	88.3
Prudhoe Bay				
(petroleum crude)	81.4	0.4	0.6	82.4
SRC-II Heavy Distillate	75.0	6.4	11.4	92.8
SRC-I Process Solvent	74.5	7.4	15.7	97.0

TABLE 10. HPLC Solvent Gradient Scheme

0 to 5 min -- 100% hexane
5 to 15 min -- linear gradient of methylene chloride in hexane
15 to 20 min -- 100% methylene chloride
20 to 30 min -- linear gradient of isopropanol in methylene chloride
30 to 35 min -- 100% isopropanol

Three major UV absorbance peaks or groups of peaks were detected in the analyses of these fractions:

1. a nonpolar peak, most prominent in the HPLC analysis of the hexane fraction
2. a "moderately polar" peak, which was seen in all three fractions but occurred mainly in the toluene/hexane and methanol fractions
3. a "polar" peak, which was the major peak detected in the methanol fraction.

When standards are run in this scheme, the "nonpolar peak" consists of aliphatic hydrocarbons and polycyclic aromatic hydrocarbons; the "moderately polar" peak includes nitrogen heterocyclics, primary aromatic amines, and carbazoles; the "polar" peak contains phenols, including nitrogen-containing phenols (Table 11). Thus, HPLC provides a promising method for separation of the abundant phenols found in the methanol fraction from the nitrogen heterocyclics and aromatic amines.

3.3.3 Accurate Mass/Metastable Ion Analysis

Biological testing of SRC liquids has shown that most of the observed genotoxicity resides in the high-boiling-point (>500°F) materials. However, the higher-molecular-weight components of high-boiling-point liquids are not amenable to gas chromatography and hence have not yet been analyzed by GCMS.

We are currently investigating the use of high-resolution probe mass spectrometry coupled with metastable ion analysis for the direct identification of higher-molecular-weight, nitrogen-containing compounds in partially purified

TABLE 11. Standards on NH₂ Column (HPLC)

<u>"Nonpolar" Region</u> <u>(0 to 9 min)</u>	<u>"Moderately Polar" Region</u> <u>(~9 to 22 min)</u>	<u>"Polar" Region</u> <u>(~22 to 35 min)</u>
Dibenzothiophene	Trimethylaniline	Phenol
Dibenzofuran	Trimethylpyridine	Naphthol
Anthracene	Quinolines	Hydroxyindole
Pyrene	Acridine	Hydroxyquinoline
Chrysene	Aminoanthracene	Aminopyridinol
Benzo[e]pyrene	Indole	Aminoquinolinol
Benzo[a]pyrene	Aminopyrene	
Nitrofluorene	Carbazole	

chemical fractions from advanced fossil fuels, including SRC-I materials. The overall strategy for such analysis includes initial screening of biologically active HPLC and TLC fractions for the presence of nitrogen-containing compounds by high-resolution or ultrahigh-resolution probe mass spectrometry, which yields data on the elemental composition of ions formed from these mixtures. Odd electron species (molecular or rearrangement ions) containing nitrogen are then selected for metastable ion analysis and the spectra thus generated are compared with those obtained from standards to determine, if possible, the functionality of the nitrogen in the molecule. Features in the metastable spectra indicative of certain fused-ring structures are also noted. Compounds thus characterized will become the object of further biotesting and analyses by independent methods to confirm their identification and to determine if they contribute to the biological activity of the coal liquid.

This approach may be very useful for the identification of biologically active, aromatic, nitrogen-containing compounds having molecular weights between about 250 and 750 amu. For heavier compounds, field desorption mass spectral analysis (FDMS) appears to be a more suitable approach. (FDMS analysis of dissolved SRC-I material, for example, indicates that some species present have molecular weights in excess of 1350 amu.)

To date, the metastable methodology, with slight modifications, has been fruitful in the analysis of SRC-II high-boiling-point liquids, having provided the first reliable data demonstrating the presence of both azabenz[a]pyrene (m/e 253) and aminobenz[a]pyrene (m/e 267), along with several alkyl homologs of both compounds. Such compounds are potentially important, since a newly synthesized member of the group (10-azabenz[a]pyrene) has been reported to be highly mutagenic in microbial assays. This work has important implications for SRC-I solid product analysis, considering the molecular weight range of the organic compounds present in this material.

Much of the remaining organic analytical work has been closely coupled with ongoing biological studies. The results of these studies will therefore be discussed in the appropriate place in Section 4.0, Biomedical Studies.

3.4 INORGANIC ANALYSES

Since a primary purpose of the solvent refining process is to produce a product that is lower in mineral content, inorganic elemental analyses of feed coal, SRC-I product, mineral residue and other process streams have been performed. Both instrumental neutron-activation analysis and X-ray fluorescence have been used to obtain an analysis of a complete spectrum of inorganic elements and to verify results obtained by the other method.

Data obtained (Table 12) show that most inorganic elements are depleted in the SRC-I product relative to the feed coal from which it was derived. For the majority of the elements, the reduction is approximately 10- to 80-fold. Exceptions were chromium, nickel, lutetium, zinc and uranium, which showed a 2- to 5-fold reduction, and titanium and bromine, for which the concentrations in feed coal and product were about equal. Analysis of mineral residue confirmed that most of the inorganic elements were being retained there.

The behavior of mercury was also different from that of other elements: its concentration in SRC-I product was lower than in feed coal, as was its concentration in the mineral residue. Balance studies (Figure 4) failed to account for about 90% of the mercury in the coal, suggesting that it was following another pathway in the process. Mass balance data for selenium,

TABLE 12. Inorganic Element Content of Solid Samples from SRC-I

	Feed Coal		Mineral Residue		Solid Product		NBS Coal Std.	
	INAA	XRF	INAA	XRF	INAA	XRF	INAA	XRF
Na	180 ± 4	3150 ± 60		8.8 ± 1.5		420 ± 20		
Cl		276 ± 30		1020 ± 50			850 ± 150	
K	1200 ± 0.02	1000 ± 100		1.16% ± 0.05	115 ± 20		0.27 ± 0.02	
Ca	--	0.069% ± 0.010		1.15% ± 0.05				0.42% ± 0.05
Ti		620 ± 20		2300 ± 200		520 ± 30	0.11 ± 0.02	0.13 ± 0.02
V	29 ± 2	25 ± 3		180 ± 20		10 ± 1	35 ± 4	38 ± 4
Cr	18 ± 2	17 ± 2	150 ± 3	161 ± 10	7.5 ± 2.1	6.0 ± 2.2	19 ± 2	18 ± 2
Mn	35 ± 2						42 ± 6	
Fe	2.29% ± 0.12	2.10% ± 0.15	13.3% ± 0.5	14.5% ± 0.3	270 ± 30	300 ± 40	0.81% ± 0.05	0.78% ± 0.035
Co	5.2 ± 0.2		30 ± 1		0.26 ± 0.04		5.2 ± 0.1	
Ni	21 ± 2	23 ± 1	120 ± 5	115 ± 5	6	2.1 ± 0.2	16 ± 2	14 ± 2
Cu		10 ± 1		126 ± 10		0.8 ± 0.1		15 ± 2
Zn	26 ± 2		120 ± 15	138 ± 20	8.1 ± 0.5	7.2 ± 0.8	37 ± 3	32 ± 3
As	19 ± 1	17 ± 2	77 ± 5	75 ± 5	3.11 ± 0.15	1.8 ± 0.2	5.7 ± 0.5	6.1 ± 0.5
Se	3.6 ± 0.2		20 ± 2	15 ± 3	0.17 ± 0.03	--	3.3 ± 0.3	3.1 ± 0.4
Br	3.6 ± 0.3	3.9 ± 0.3	4.9 ± 0.4	5.2 ± 0.4	4.7 ± 0.4	4.8 ± 0.4	17 ± 2	17 ± 2
Rb	13 ± 1	15 ± 1	92 ± 10	96 ± 5		0.4	19 ± 2	18 ± 1
Sr	59 ± 15	140 ± 10	310 ± 30	325 ± 15	0.96 ± 0.16	2	170 ± 20	150 ± 20
Sb	1.4 ± 0.1		7.4 ± 0.3		0.066 ± 0.009		3.7 ± 0.3	
Ba	46 ± 5		240 ± 25					
La	8.9 ± 0.5		46.8 ± 1.2					
Hf	0.44 ± 0.02		2.3 ± 0.2		0.05 ± 0.005		0.97 ± 0.09	
Hg	0.16 ± 0.01		.005		0.020 ± 0.005		0.12 ± 0.02	
Pb		8 ± 1		42 ± 3		1		11
Th	1.9 ± 0.1		9 ± 1		0.19 ± 0.03		3.4 ± 0.3	
U	1.1 ± 0.1		7.3 ± 0.3		0.54 ± 0.05		1.6 ± 0.2	

arsenic, titanium, iron and bromine are also shown in Figure 4. For these elements, recovery was 80% or greater, which further suggests that the mercury was not being carried with the product, light oil or mineral residue.

Data for sulfur are not given in the figure. However, other analyses have shown that the SRC-I product has an approximately 10-fold reduction relative to the feed coal. The major fraction is recovered as elemental sulfur and does not appear in the mineral residue.

Data obtained for the liquid process streams, light oil, and process solvent (Table 13) show that they have relatively low concentrations of the inorganic elements. These determinations did not include sulfur, but analysis of the naphtha and heavy distillate from the SRC-II process show that they contain about 1600 and 3600 ppm of sulfur, respectively.

FIGURE 4. Balances for Six Elements in a Coal Liquefaction-Refined Solid Process

TABLE 13. Inorganic Element Content of Liquid Samples from the SRC-I Process (in ppm except as noted)

	Light Oil		Process Solvent	
	INAA ^(a)	XRF ^(b)	INAA	XRF
Na	1.7 $\pm$ 0.03		0.51 $\pm$ 0.01	
Cl		<36		
K		<3		<67
Ca		<3		
Ti	1.8 $\pm$ 0.7	2.2 $\pm$ 0.15		9.3 $\pm$ 3.1
V		<6		<3.2
Cr	0.044 $\pm$ 0.009	<0.2		
Mn		0.21 $\pm$ 0.06		<1.11
Fe	1.1 $\pm$ 0.3	<2	58 $\pm$ 2	102 $\pm$ 7
Co	0.0026 $\pm$ 0.0005		0.0044 $\pm$ 0.0001	
Ni	0.10	<0.05		1.35 $\pm$ 0.2
Cu		<0.07		<0.4
Zn	0.58 $\pm$ 0.04	0.46 $\pm$ 0.07	0.86 $\pm$ 0.04	0.54 $\pm$ 0.14
As	0.013 $\pm$ 0.001	0.01 $\pm$ 0.02	0.105 $\pm$ 0.002	<0.23
Se	0.065 $\pm$ 0.020	0.086 $\pm$ 0.030	<0.009	<0.15
Br	0.018 $\pm$ 0.002	<0.07	0.25 $\pm$ 0.01	0.29 $\pm$ 0.05
Rb	0.05		<0.06	<0.26
Sr		<1.1		<2.4
Sb	<0.002		0.006 $\pm$ 0.09	
Ba	<1		<2	
La	<0.001		0.0125 $\pm$ 0.008	
Hf	<0.003		<0.003	
Hg	0.05 $\pm$ 0.01		<0.01	
Pb		<0.1		<1.7
Th	<0.0009		0.001	

(a) Instrumental neutron activation analysis.

(b) X-ray fluorescence.

4.0 BIOMEDICAL STUDIES

The general philosophy regarding our approach to biomedical studies with SRC materials has been discussed in an earlier status report (PNL-3189). In brief, we select materials for study on the basis of quantities produced, the likelihood of exposure, the chemical composition, and the relevance to large-scale operation. The materials selected are subjected to biological study in two concomitant modes. In the first, materials are evaluated using a tiered approach. That is, they are first screened for various kinds of genetic activity and cellular toxicity using microbial systems, such as the Ames Salmonella assay. On the basis of the results of the microbial assays, materials are selected for study in mammalian cell culture, a higher-order system. Results generated in the cellular models are used as selection criteria for materials to be used in animal assays, particularly those with carcinogenesis as an end point.

In the second mode, materials considered to be of prime importance with regard to occupational or environmental health are entered directly into animal systems for study of acute, subchronic and developmental toxicity. Using the results of these studies and of the cellular assays together with the chemical characterization data, long-term effects studies are designed. At each level of testing, we have included other materials for comparative purposes. These materials, which include shale oil, petroleum crudes and other fossil-derived materials, as well as pure forms of known chemical mutagens and carcinogens, help to place results obtained with SRC materials in a clearer perspective.

In the following sections, the results of a number of biological studies with SRC and other materials are summarized. These studies deal with:

1. microbial mutagenesis/chemical characterization
2. in vitro mammalian-cell toxicity and transformation
3. acute and subchronic oral toxicity
4. developmental toxicity
5. aerosol generation and inhalation exposure, and
6. dosimetry and metabolism.

4.1 MICROBIAL MUTAGENESIS/CHEMICAL CHARACTERIZATION

The use of cellular systems, both microbial and mammalian-cell cultures, permits the study of a large number of materials at relatively low cost. The most commonly employed cellular assay is the Ames Salmonella mutagenesis assay. This assay is currently being used extensively because it is simple, rapid, low cost, requires small amounts of test materials, and has a high correlation with carcinogenicity in the case of pure compounds. At this time, a number of SRC and other materials used for comparative purposes have been assayed by standard Ames methods or by modifications which optimize the mutagenic evaluation of complex chemical mixtures. Materials found to be active in the Ames system have been fractionated by chemical and physical means in order to determine which components are responsible for mutagenic activity.

Briefly, the Ames assay is performed by mixing the test material with the organism in the presence or absence of mammalian liver microsomal enzymes (S9) and determining the number of organisms which revert (mutate) from dependency on histidine in the medium to nondependency. Assay in the presence of S9 detects mutagens which require metabolic activation to effect mutagenesis; assay in the absence of S9 detects mutagens which do not require metabolic activation. Use of appropriate strains of Salmonella permits determination of the nature of the mutation (frame shift or point mutation), which is a function of the biochemical properties of the test materials.

Examples of the data obtained with a number of materials are shown in abbreviated form in Table 14. Of the three SRC-I materials (LO, WS and PS), only the PS showed activity in the Ames system. Process solvent was about 1/10 as active as benzo[a]pyrene but 20 times more active than Paraho shale oil. Crude Prudhoe Bay petroleum showed no activity in the Ames assay.

Fractions prepared by solvent extraction of PS were assayed in the Ames system. The results shown in Table 15 indicate that the basic fraction had the highest specific activity (revertants/ μ g) of the fractions tested, although more total activity was associated with the tars because of their greater mass. Qualitatively similar results were obtained with fractions prepared from crude shale oil.

TABLE 14. Comparison of the Mutagenicity of Solvent Refined Coal Materials, Shale Oils, and Crude Petroleums in Salmonella Typhimurium TA98

<u>Materials</u>	<u>Revertants/μg Material</u>
SRC-I	
Process solvent	12.3 ± 1.9
Wash solvent	<0.01
Light oil	<0.01
Shale Oil	
Paraho-16	0.60 ± 0.19
Paraho-504	0.59 ± 0.13
Livermore L01	0.65 ± 0.22
Crude Petroleum	
Prudhoe Bay	<0.01
Wilmington	<0.01
Pure Carcinogens	
Benzo[a]pyrene	114 ± 5
2-Aminoanthracene	5430.65 ± 394

These results led to further studies of the nature of mutagenic materials present in PS. Results of previous work on coal liquids had yielded evidence that polar nitrogen-containing compounds, in particular the primary aromatic amines (PAAs), might be present in this higher boiling SRC distillate. To test this idea, the most mutagenically active of the fractions derived from PS, the basic and tar fractions, were analyzed by thin-layer chromatography (TLC) using a solvent system designed to separate the polar nitrogen-containing compound classes known or suspected to be present in HD. Fluorescent areas on the TLC plates corresponding to the relative mobilities of these chemicals were cut into strips and the strips extracted with a hexane-acetone mixture. The extracts were then studied for mutagenic activity and also analyzed for chemical composition by GCMS.

In brief, aliquots of the TLC eluates were adjusted to a volume of 300 μ l after adding 100 μ g of d₁₀ anthracene, which served as an internal standard.

TABLE 15. Ames Assay Comparison of SRC-I Fractions Versus Shale Oil Fractions

Fractions	Revertants/ μ g for <i>S. typhimurium</i> ^(a)			
	TA 98	TA 100	TA 1538	TA 1535
SRC-I Process Solvent Basic	155 + 58 ^(b) (0.5 to 10)	11 + 61 ^(c) (50 to 300)	102.1 + 19.4 (10 to 160)	<0.1
SRC-I Process Solvent Basic Tar	24 + 2.1 (0.5 to 11)	0.8 + 6.8 (50 to 500)	9.9 + 8.2 (10 to 160)	<0.1
SRC-I Process Solvent Neutral Tar	32.1 + 10.4 (0.5 to 11)	4.6 + 1.6 (50 to 350)	11.6 + 5.6 (10 to 160)	<0.1
Paraho-16 Basic	2.1 + 0.6 (15 to 130)	2.1 + 0.4 (50 to 250)	— ^(d)	
Paraho-16 Tar	1.0 + 0.4 (30 to 600)	2.4 + 0.9 (50 to 200)		
Paraho-504 Basic	4.0 + 0.4 (15 to 130)	1.7 + 0.6 (60 to 300)		
Paraho-504 Tar	0.2 + 0.1 (30 to 600)	0.5 + 1.4 (60 to 300)		
Livermore L01 Basic	2.9 + 0.9 (15 to 130)	1.6 + 0.4 (50 to 250)		
Livermore L01 Tar	0.4 + 3.3 (30 to 600)	2.1 + 0.2 (50 to 250)		
2-Aminoanthracene				
SRC-I	5430 + 394 ^(e)			
SO	7886 $\pm$ 1196			
Benzo[a]pyrene				
SRC-I	123 + 7			
SO	202 $\pm$ 25			

(a) All results for each assay were normalized to 1×10^8 cells of the indicated *S. typhimurium* test strain.

All products were assayed with sufficient S9 protein to yield maximum mutagenic responses for the various materials.

Background revertant colonies for the four test strains were: TA 98, 19; TA 100, 192; TA 1535, 50; TA 1538, 10. These values were subtracted from the data in the table.

- (b) The numbers, plus and minus standard deviations, represent the slope of the response curves expressed as revertants/ μ g/plate. Each concentration was represented by four identical samples, which were averaged.
- (c) Where the mutagenic response was nonlinear, the high and low values for mutation are given. The concentration range (as above) is given in parentheses.
- (d) Not assayed.
- (e) Values for positive controls (2-aminoanthracene and benzo[a]pyrene) were determined in the same experiments involving the synfuel process materials. Designation in parentheses refers to synfuel material evaluated versus the positive control; SRC is solvent refined coal; SO is shale

They were then subjected to GCMS analyses using a 25-m x 0.25-mm ID SE-52 phase WCOT capillary column interfaced to a mass spectrometer via a platinum-iridium capillary. Relative concentrations for the several compounds of interest in the samples were calculated by comparing the areas of the compound molecular ion peaks to that of the d₁₀ anthracene in computer-generated, selected ion chromatograms. Although such methods have sufficient precision to allow absolute concentration estimates when the relative ionization efficiency of each compound is known, they are used here for comparison of relative concentrations only.

In these experiments, both mutagenically active and inactive TLC regions were screened for PAA and selected azaarenes of similar structures. Association of PAA with genetic activity in the TLC fractions of PS was assumed to constitute a necessary condition if PAA were important determinants of mutagenicity in this material.

4.1.1 Basic Fraction

The mutagenic activity recovered by TLC from the PS basic fraction was localized entirely in TLC regions (1 + 2) and (3) (Figure 5). The 2-, 3- and 4-ring polyaromatic amines (PAAs), the aminonaphthalenes (AN), aminoanthracenes (AA) + aminophenanthrenes (APH) and aminopyrenes (AP) were tightly clustered in these TLC regions (Figure 6). Moreover, their concentrations in the eluates from TLC regions (1 + 2) through (5 + 6) increased and decreased with increases and decreases in mutagenicity.

Although the azaarenes overlapped mutagenic TLC regions, they were also found in nonmutagenic TLC regions (Figure 6). For example, more than 40% of the quinolines, greater than 50% of the benzoquinolines, and about 40% of the azapyrenes were found outside TLC regions (1 + 2) and (3). The carbazoles also overlapped the mutagen-containing TLC regions, with about 60% of these compounds found outside the regions (1 + 2) and (3) (Figure 7).

4.1.2 Basic Tar Fraction

The mutagenic activity recovered after TLC of the PS basic tar fraction was distributed among TLC regions (1 + 2), (3) and (4 + 5) in approximately

FIGURE 5. Fractionation of the Mutagenic Activity in the Basic, Basic Tar and Neutral Tar Fractions of Process Solvent Using Thin Layer Chromatography

FIGURE 6. Relative Concentrations of Several N-Containing Compounds in Various Regions of a Thin Layer Chromatogram of the Basic Fraction of Process Solvent

FIGURE 7. Carbazole Content of Various Regions of Thin Layer Chromatograms of Basic, Basic Tar and Neutral Tar Fractions of Process Solvent

equal amounts (Figure 5). As for the PS basic fraction, the PAAs in this tar were localized entirely in the mutagenically active TLC regions (Figure 8). But in contrast to the PS basic fraction, the 2- and 3-ring PAAs in this tar fraction were much lower in concentration relative to the 4-ring APs. The 2-ring ANs, for example, were barely detectable above background by GCMS analysis.

The 2- and 3-ring azaarenes from the basic tar fractions of PS were found in both mutagenic and nonmutagenic TLC regions. For example, approximately 43% of the quinolines and about 30% of the benzoquinolines in the basic tar fraction were associated with nonmutagenic TLC regions. However, the azapyrenes were concentrated mainly in TLC region (4 + 5), which contained a significant portion of the mutagenicity of this material.

The carbazoles from the PS basic tar fraction were concentrated mainly in TLC regions (3), (4 + 5) and (6 + 7), with smaller amounts in (8 + 9) (Figure 7). Approximately 24% of these compounds were found outside the mutagenic TLC regions.

4.1.3 Neutral Tar Fraction

The mutagenic activity from the PS neutral tar fraction was localized primarily in TLC regions (1) and (2 + 3), with approximately 10% in regions (4 + 5) (Figure 5). The PAA were found only in mutagenically active TLC eluates, in concentrations roughly proportional to the total mutagenic response associated with these eluates (Figure 9). The PS neutral tar fraction, in common with the basic tar fraction, contained very low concentrations of the aminonaphthalenes, but relatively high concentrations of the 4-ring aminopyrenes.

The 2-, 3- and 4-ring azaarenes were broadly distributed across mutagenic and nonmutagenic TLC regions. For example, more than 80% of the quinolines and benzoquinolines were found in TLC regions containing 10% or less of the mutagenic activity recovered from the neutral tar fraction.

The carbazoles recovered in the PS neutral tar fraction were distributed as follows: approximately 38% in TLC regions (2 + 3) and 62% in regions (4 + 5) and (6 through 9), i.e., essentially outside zones of mutagenic activity (Figure 7).

FIGURE 8. Relative Concentrations of Several N-Containing Compounds in Various Regions of a Thin Layer Chromatogram of the Basic Tar Fraction of Process Solvent

FIGURE 9. Relative Concentrations of N-Containing Compounds in Various Regions of a Thin Layer Chromatogram of the Neutral Tar Fraction of Process Solvent

There is an interesting difference between the PAA constituents of SRC-I basic fraction and the two tar fractions. The former contained relatively large concentrations of the 4-ring aminopyrenes and of AN. The tars, on the other hand, contained only trace amounts of the aminonaphthalenes and relatively lower concentrations of the 3-ring aminoanthracenes and aminophenanthrenes. Since the tars constitute approximately 20% to 40% by weight of the PS, and the basic fraction is roughly 2%, by weight, of PS, the dominant PAA species of this material would appear to be the aminopyrenes, although the pyrene ring structure itself is much less abundant than the 2- and 3-ring species in the liquid as a whole.

4.2 MAMMALIAN CELL-CULTURE STUDIES

A second step in the tier approach to evaluating the biological effects of SRC materials is to test for their ability to cause cultured mammalian cells to undergo morphological and biochemical changes (transformation). In vitro transformation provides further presumptive evidence of the potential carcinogenicity of a test material.

We have used Syrian hamster embryo (SHE) cells to study the ability of SRC materials to effect transformation. In this assay, second-passage cells are preincubated in petri dishes for 16 to 24 h, then treated with the test material, either with or without S9 to provide metabolic activation. After 21 days, the cells are fixed and stained and the number of transformed colonies, as well as the total number of colonies, is counted. In some cases, transformed cells are inoculated into nude mice to evaluate their tumor-forming ability in vivo.

A comparison is shown in Figures 10a and b of the effects of BaP and PS. Both materials produce a moderate degree of toxicity in addition to transformation. The dose-response curves for both transformation and survival are steeper for BaP than for PS, although the maximum response for both end points is similar for both materials.

For most materials tested, the presence of S9 was necessary to effect transformation (Table 16). However, a small amount of activity was observed with PS and the basic fraction of PS without S9, indicating the presence of direct-acting agents.

FIGURE 10a. Effect of BaP on Survival and Transformation of Syrian Hamster Embryo (SHE) Cells. The test plates contained secondary cultures of Syrian hamster embryo cells, various concentrations of test chemical and S9 activating enzymes (7 mg of protein). Cell survival was determined at 7 days postexposure and transformation frequency at 20 days postexposure. Each data point represents the summation of six plates. Colonies were scored blind.

FIGURE 10b. Effect of Process Solvent on Survival and Transformation of Syrian Hamster Embryo (SHE) Cells. The test conditions were the same as for Figure 10a.

TABLE 16. Effect of Microsomal (S9) Activating Enzymes on Transformation Frequency of Syrian Hamster Embryo (SHE) Cells Exposed to Complex Organic Mixtures

Test Material, Concentration in $\mu\text{g/ml}$	Activating (S9) Enzymes	Relative Survival	Percent Transformation
DMSO (Control)	+	91	0
	-	100	0
Prudhoe Bay Crude (200)	+	24	0.5
Shale Oil-LERC (10)	+	39	3.1
	-	92	0
Shale Oil Basic Fraction (50)	+	48	7.1
	-	55	0.5
Shale Oil PNA Fraction (10)	+	52	3.9
	-	88	0
SRC-I Process Solvent (50)	+	31	6.8
	-	43	1.1
SRC-I Process Solvent Basic Fraction (10)	+	52	12.8
	-	92	0.8
SRC-I Process Solvent PNA Fraction (50)	+	52	3.9
	-	68	0
Benzo[a]pyrene (5)	+	75	4.8
	-	84	0

A comparison of the transforming activities of a number of materials is shown in Table 17. Process solvent, shale oil and HD had transforming activities ranging from 20 to 100% that of BaP. The transforming activities of the basic and PNA fractions of PS and SO were higher than for the parent mixture. The petroleum crudes also showed a low level of activity in this assay. In contrast, neither petroleum crudes nor the PNA fraction of PS was active in the Ames system. A few cell lines derived from PS-transformed colonies have been inoculated into nude mice. All produced undifferentiated sarcomas at the site of inoculation, suggesting that these transformed cells are neoplastic.

TABLE 17. Comparative Transformation Properties of Complex Organic Mixtures

Material	Number of Transformants per μg of Test Material(a)	Range of Linear Portion of Concentration Response Curve(b)
DMSO Control (0.1%)	0	--
Prudhoe Bay Crude	~ 0.002	100 to 200
Wilmington Crude	~ 0.002	75 to 150
SRC-II Heavy Distillate	0.7	5 to 20
SRC I Process Solvent	0.15	5 to 40
Basic Fraction	1.2	1 to 20
PNA Fraction	0.3	1 to 5
Shale Oil (LERC)	0.3	1 to 10
Basic Fraction	0.76	1 to 20
PNA Fraction	0.8	5 to 50
Benzo[a]pyrene	0.76	1 to 20

(a) Extrapolated from linear region of the concentration response curve

(b) $\mu\text{g/ml}$

None of the SRC-I materials has yet been subjected to skin-painting assays for carcinogenicity. However, in studies reported elsewhere (PNL-3184), HD, SO, Wilmington crude and BaP have been shown to be carcinogenic. Since PS produces a strong positive response in the microbial mutagenesis and mammalian cell assays, as did the materials previously listed, it seems very likely that PS may also be carcinogenic in the skin-painting assay.

4.3 ACUTE AND SUBCHRONIC ORAL TOXICITY

The toxicities of SRC-I LO, WS and PS have been compared to those for SRC-II LD, MD, HD and SO, petroleum crudes, and diesel oil (DO) after oral administration. These studies were performed to provide estimates of the limits of tolerance for these materials and to guide the selection of doses to be used in studies of developmental toxicity. The test material was administered by stomach tube, either undiluted or diluted in corn oil, to adult Wistar rats

which had been fasted overnight. Animals were observed closely for 3 days following administration of the test material for clinical symptoms of toxicity. Animals were weighed before and for 2 weeks following dosing. Process solvent and HD have also been studied in immature 14- and 21-day-old rats to determine if the younger animal is more sensitive.

The relative toxicities of these materials, expressed as the dose (g/kg body weight) required to kill 50% of the animals in 3 days, are shown in Table 18. Wash solvent was clearly the most toxic of the materials that we examined ($LD_{50} = 0.57$ g/kg). The LD_{50} values of LD, PS, LO, HD and MD formed a group ranging from 2.3 to 3.8 g/kg; the values for SO, DO, and crude petroleum were in excess of 9.0 g/kg. If these data for SRC materials were directly extrapolated to humans, the materials would be considered moderately toxic (rating of 3 on a scale of 1-6, as used in Clinical Toxicology of Commercial Products, 1976).

Fourteen-day-old rats were significantly more sensitive to PS ($LD_{50} = 1.74$ g/kg) and HD ($LD_{50} = 1.41$ g/kg) than were the adults. Weanlings (21 days old) were only slightly more sensitive than the adults.

Subchronic toxicity was determined for LO, WS, PS, LD, MD and HD by administering the material by gavage once daily to fasted female Wistar rats, for 5 consecutive days. Death, weight changes and clinical symptoms of toxicity were monitored as in the acute studies.

The subchronic LD_{50} values for LO and WS were not greatly different from those of the acute studies (Table 18). The subchronic values for all of the other materials were substantially lower than the acute values. These data indicate that the cumulative effects of LO and WS are rather low but that for PS, LD, MD and HD either the material or the effects are cumulative.

4.4 DEVELOPMENTAL TOXICITY

Since the prenatal individual is significantly more sensitive to a number of toxic agents than are adults, studies were performed to determine the effects of SRC-I materials administered to rats during pregnancy. The study materials were administered once daily for 5 consecutive days at each of two different periods: 7 to 11 and 12 to 16 days of gestation (dg). The first

TABLE 18. Comparison of the Acute and Subchronic Toxicities of SRC Materials in the Female Wistar Rat^(a)

<u>Material</u>	<u>Acute LD₅₀^(b)</u>	<u>Subchronic LD₅₀^(b)</u>
Wash solvent	0.57 (1.66) ^(c)	(1.50) ^(c)
Light distillate	2.30	0.96
Process solvent	2.81	1.04
Light oil	2.90	2.41
Heavy distillate	2.98	1.19
Middle distillate	3.75	1.48
Shale oil	9.22	----
Diesel oil	11.8	----
Crude petroluem	12.0	----

- (a) The materials were administered one time by gavage, for the acute toxicity studies. For the subchronic studies, materials were gavaged once daily for 5 consecutive days.
- (b) LD₅₀ is defined as the dose in grams per kilogram of body weight required to kill 50% of the animals. For the acute toxicity study, this value represents a single dose; for the subchronic studies, the value represents a daily dose.
- (c) The LD₅₀ values in parentheses are for materials diluted in corn oil. All other values are for undiluted material.

period represents early organogenesis, when organs are beginning to form; the second represents a period of rapid growth and early maturation. Dosages ranged from those producing significant maternal toxicity (2.9 g/kg/day) to those producing no apparent effect in dam or fetus (0.2 g/kg/day). Corn oil alone (3 ml/kg/day) was administered to vehicle control groups; a polychlorinated biphenyl (Aroclor 1254, 2.5% in corn oil) was used for positive control groups. All animals were fasted overnight prior to agent administration. Maternal toxicity was estimated in pregnant and nonpregnant female Wistar rats dosed from 7 to 11 or 12 to 16 dg. Rats were sacrificed at 21 dg for evaluation of prenatal mortality (resorptions), intrauterine growth retardation (IUGR) and teratogenesis (malformations).

The detailed data for administration at 7 to 11 or 12 to 16 dg are presented in Tables 19 and 20, respectively. Only the most noteworthy observations are indicated in the following paragraphs.

Administration of LO consistently induced IUGR only at doses that were both maternally toxic and lethal. Prenatal mortality was elevated only after dosing with maternally toxic doses from 12 to 16 dg. Occasional major malformations were seen in fetuses of some groups dosed from 7 to 11 dg, but not after dosing from 12 to 16 dg.

Administration of WS did not induce IUGR nor a significant incidence of malformations at doses that were not also maternally toxic and lethal. The incidence of prenatal mortality was elevated only following dosing at 12 to 16 dg.

Administration of PS induced prenatal mortality at doses below those that were maternally toxic, but generally prenatal mortality was accompanied by signs of maternal toxicity. The occurrence of IUGR and major malformations increased only following dosing from 12 to 16 dg. Cleft palate was the malformation most often seen.

Thus, dose-related manifestations of developmental toxicity were rarely seen at doses lower than those inducing maternal toxicity as well. Dosing from 12 to 16 dg tended to produce more maternal and fetal toxicity than did dosing from 7 to 11 dg.

4.5 INHALATION STUDIES

Since inhalation represents a likely route of exposure of occupational personnel to coal liquids, we have been developing methods for generating and characterizing respirable aerosols of PS and for exposing experimental animals to such aerosols. Preliminary toxicity studies (up to 3 weeks of exposure) have also been performed.

After considering several generating systems, we chose a Retec® nebulizer with a glass reservoir (PS attacks the usual polystyrene reservoir).

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TABLE 19. Maternal and Fetal Toxicity in Rats Following Dosing with SRC-I Materials from 7 to 11 Days of Gestation

Agent	Maternal Considerations					Fetal Considerations				
	Dose, g/kg/day(a)	Number Dosed(c)	Percent Dead(c)	Number Pregnant at 21 dg	Weight Gain, kg to 21 dg(d)	Percent Implants Resorbed(e)	Litters with Resorptions	Percent Malformed Fetuses(e,g)	Litters with Malformations(g)	Fetal Weight, kg at 21 dg(h)
Corn Oil	--	14	0	13	154 ± 22	6	5	1	1	5.6 ± 0.4
Aroclor ^(b)	0.08	5	0	4	96 ± 30	41	4	0	0	4.9 ± 0.6
Light Oil ^(b)	0.24	4	0	2	164 ± 1	7	2	0	0	6.2 ± 0.4
	0.72	4	0	3	147 ± 5	0	0	2	1	5.9 ± 0.5
	1.20	4	0	3	178 ± 4	0	0	0	0	5.8 ± 0.3
Light Oil	2.39	9	56	2	110 ± 50	3	1	0	0	4.9 ± 0.7
	2.63	5	80	1	88	8	1	9	1	4.2 ± 0.4
	2.87	5	80	0	--	--	--	--	--	--
Wash Solvent ^(b)	0.85	6	0	6	139 ± 22	5	3	0	0	5.7 ± 0.5
	1.11	5	0	5	144 ± 22	5	3	0	0	5.4 ± 0.4
	1.27	7	0	4	124 ± 41	6	2	0	0	5.9 ± 0.6
	1.43	9	0	5	114 ± 33	16	3	2	1	5.1 ± 0.4
	1.58	6	67	2	154 ± 2	4	1	0	0	5.4 ± 0.2
Process Solvent ^(b)	0.34	8	0	6	150 ± 23	22	5	0	0	5.9 ± 0.5
	0.51	8	0	6	127 ± 29	25	5	0	0	5.6 ± 0.5
	0.69	8	0	5	88 ± 37	75 ^(f)	5	6	1	6.0 ± 0.5
	0.85	5	0	4	52 ± 38	77 ^(f)	4	0	0	5.3 ± 0.7

(a) Administered by gavage once daily for 5 consecutive days; if undiluted, 0.1 to 1.2 ml given per 300 g body weight.

(b) Diluted in corn oil and 1 ml given per 300 g body weight.

(c) Includes both pregnant and nonpregnant adult females.

(d) Body weight gain between 0 and 21 days of gestation (dg); mean ± SD

(e) Calculated on a per fetus rather than a per litter basis; includes resorbed and dead implants.

(f) One or more litters with all implants resorbed

(g) Combination of soft tissue and skeletal malformations

(h) Pooled means of each litter

TABLE 20. Maternal and Fetal Toxicity in Rats Following Dosing with SRC-I Materials from 12 to 16 Days of Gestation

Agent	Maternal Considerations					Fetal Considerations				
	Dose, g/kg/day(a)	Number Dosed(c)	Percent Dead(c)	Number Pregnant at 21 dg	Weight Gain, kg to 21 dg(d)	Percent Implants Resorbed(e)	Litters with Resorptions	Percent Malformed Fetuses(e,g)	Litters with Malformation(g)	Fetal Weight, kg at 21 dg(h)
Corn Oil	--	22	0	20	141 ± 30	2	4	1	2	5.5 ± 0.5
Aroclor(b)	0.08	14	0	11	116 ± 30	12	8	5	2	4.6 ± 0.5
Light Oil	1.44	5	0	3	135 ± 18	7	3	0	0	5.2 ± 0.4
	1.69	10	20	7	109 ± 34	5	2	0	0	5.1 ± 0.4
	1.93	13	0	7	58 ± 57	29	6	0	0	3.9 ± 0.8
	2.17	10	30	5	84 ± 40	8	4	0	0	4.6 ± 0.7
	2.41	11	27	5	83 ± 81	35(f)	3	0	0	4.7 ± 0.7
Wash Solvent(b)	0.95	13	0	10	118 ± 43	14	4	0	0	5.4 ± 0.5
	1.11	10	0	7	98 ± 39	10	3	2	1	4.7 ± 0.7
	1.27	9	67	2	88 ± 56	50	1	0	0	5.0 ± 0.3
Process Solvent(b)	0.34	5	0	3	128 ± 3	10	2	6	1	5.0 ± 0.4
	0.52	5	0	4	100 ± 28	24(f)	4	4	1	4.8 ± 0.5
	0.69	13	8	10	86 ± 32	28	7	13	6	4.4 ± 0.6
	0.85	12	25	6	55 ± 41	70(f)	6	13	2	4.3 ± 0.8
	1.03	4	50	1	72	75	1	33	1	3.4 ± 1.0
Process Solvent	0.34	6	0	4	134 ± 41	5	2	0	0	5.2 ± 0.4
	0.69	6	0	6	93 ± 41	36	3	4	2	4.8 ± 0.7
	1.03	3	67	1	53	54	1	50	1	3.4 ± 0.4

(a) Administered by gavage once daily for 5 consecutive days; if undiluted, 0.1 to 1.2 ml given per 300 g body weight.

(b) Diluted in corn oil and 1 ml given per 300 g body weight.

(c) Includes both pregnant and nonpregnant adult females.

(d) Body weight gain between 0 and 21 days of gestation (dg); mean ± SE

(e) Calculated on a per fetus rather than a per litter basis; includes resorbed and dead implants.

(f) One or more litters with all implants resorbed

(g) Combination of soft tissue and skeletal malformations

(h) Pooled means of each litter

Initially, the aerosol was generated into a 1-m³ plexiglass chamber and periodic samples were taken from several areas of the chamber for determination of concentration. Other samples were taken with a Mercer® cascade impactor to determine the range of particle sizes.

Since quantitative analysis by gas chromatography is a long and cumbersome procedure, a more rapid method was sought. We found that PS showed an absorbance peak at 254 nm (Figure 11). A standard curve was prepared and used for calculations of aerosol concentrations.

Aerosol samples were obtained from the chamber by withdrawing air through a Millipore® filter (type HAWP, 0.45 μ m) with a 1.0-l syringe. The sampling

FIGURE 11. Ultraviolet Absorption Spectrum of Process Solvent in Chloroform

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time was approximately 45 sec. The filter containing the aerosol was transferred to a glass vial containing 10 ml of chloroform, and the absorbance of the solution was measured at 254 nm.

Initially, the production of aerosol with the Retec nebulizer was highly variable. This variability was found to be related to the volume of PS in the reservoir and to clogging of the nebulizer siphon tube. These problems were eliminated by filtering the PS through a glass-fiber filter and maintaining larger amounts of PS in the reservoir. Aerosol concentrations of 1 to 2 mg/l were consistently achieved when these actions were taken. The mass median aerodynamic diameter of the aerosols ranged from 1.4 to 2.1 μm .

In the first animal experiment, 12 rats were exposed for 6 hr/day for 5 days to an average aerosol concentration of 6.3 mg/l of PS. The most pronounced symptoms were profuse tearing of the eyes and loss of hair, which sometimes fell out in patches. By the end of the exposure period, each animal had lost an average of 30 g of body weight, a 10% reduction.

In the second experiment, 12 animals were exposed for 3 wk, 6 hr/day, 5 days/wk. The average concentration of PS in the aerosol over the 3-wk period was 1.2 mg/l, with an average mass median aerodynamic diameter of 2.2 μm . The toxic symptoms, which were similar to those observed in the first experiment, included reduced body weight, eye irritation and loss of hair. During the early portion of exposure, the animals as a group showed a very rapid weight loss which plateaued in the second week. On the last day of exposure, individual animals had lost from 2 to 29% of their pre-exposure weights. The group mean was significantly reduced ($p < 0.01$). The animals fell into three arbitrary but distinct weight-loss groups: <6% loss, three animals; 9 to 13% loss, five animals; >18% loss, four animals. By 30 days after completion of exposure, all animals were within 3% of their beginning weights. The animals were then killed and lungs, liver, kidneys, gastrointestinal tract and skin were examined. No grossly observable lesions were detected. The lungs contained dark, diffuse spots that were presumably SRC-derived material; however, this observation awaits histological confirmation.

These experiments demonstrated the feasibility of generating high concentrations of respirable particles of PS into a small chamber for exposure of a limited number of animals. Since longer-term experiments (90 days to 2 years) require large groups of animals, we began to adapt the generating system to provide suitable concentrations of aerosol for a Battelle-designed chamber (approximately 87 ft³). To provide suitable amounts of material at the highest desired concentration (nominally 1 mg/l), four Retec nebulizers were connected by a manifold to a common reservoir. The aerosol passed from the reservoir to the chamber through a 1-in. (ID) polyethylene tube.

The concentration of aerosol was determined at various positions in the chamber by drawing measured volumes of air through a Millipore® filter. The PS was eluted into chloroform and the absorbance determined as described. Mercer cascade impactor samples were also routinely obtained for determination of particle size. The particle size distribution was calculated using the FORTRAN program "NEWCAS."

A single Retec generator under the described chamber conditions produced an aerosol concentration of 0.16 mg/l. Increasing the number of generators to four produced an average aerosol concentration of 0.60 mg/l. Concentrations lower than 0.16 mg/l were obtained by diverting a portion of the aerosol into the exhaust system.

Relatively stable aerosol concentrations were maintained in the chamber throughout a 6-h exposure period. However, over the first few days of generation the aerosol concentration declined consistently. We found that chloroform, which had been used for daily rinsing, was affecting the disassembled plastic generator parts. When a new generator was used each day, average aerosol concentration was approximately that desired; however, daily variability was larger than desired. Most recently, we have thoroughly rinsed the generator in methanol, without disassembly. Figure 12 is a plot of the daily aerosol concentration during a recent 13-day exposure. The variability within a given day, which was routinely determined by collecting six samples, taken in pairs, from the same location at 1, 3 and 5 h of generation, had an average coefficient of variation⁽²⁾ of 0.062. Since the coefficient of variation for the

FIGURE 12. Concentration of Process Solvent Aerosol in the Animal Exposure Chamber During a 13-day Test Period

sampling technique was 0.061, it is apparent that the major portion of the variability is in the sampling procedure. The sampling variability probably reflects the short time (45 sec) involved in collecting each sample. The source of the day-to-day variation, which was greater than the daily variation, was in the generator.

A potential source of error within the analytical procedure was the background absorption from the Millipore filters. These filters contain an organic binder that has a broad absorption spectrum in the UV range. The background absorption (optical density) average 0.15 was inconsistent from one batch of filters to the next. We therefore purchased sufficient filters from a single batch and daily determined the background absorption. In order to reduce the chance for variability from background absorption, sufficient aerosol was collected to give an absorption at least four times that of the filter.

The PS contained small particles (probably SRC material) that precipitated during storage. These particles sometimes plugged the generator orifice which caused the aerosol concentration within the chamber to decrease. To avoid this problem, the PS was routinely filtered through glass wool.

Although absorption at 254 nm was routinely used to determine chamber concentrations and particle size distribution for PS aerosols, it appeared necessary to verify that the components of PS were not being fractionated during passage of the aerosol through the chamber. Therefore, samples of aerosol were collected with a Mercer cascade impactor using glass stages weighed to 0.1 μg with a microbalance. The material present on the impactor stages was rinsed into a vial with chloroform containing the internal standards, decane and eicosane (approximately 0.5 mg/mL each). In addition, a fitted solvent trap containing chloroform was placed immediately downstream from the impactor to collect organic vapors. The solvent trap solution and the rinses from the impactor stages were concentrated to approximately 100 μL with a gentle stream of nitrogen at room temperature. These samples were subjected to quantitative analysis for 11 major components using the gas chromatographic method described in Section 3.2. Samples of PS from the Retec reservoir, taken after aerosol generation, were analyzed for comparison with the stock PS as a further check for possible nonuniform aerosolization of PS constituents.

Data presented in Table 21 indicate that the concentrations of the components measured varied with the impactor stage. The concentration of the lower-molecular-weight materials, naphthalene, 1- and 2-methylnaphthalene and biphenyl, was generally higher in the smaller particles (note filter values); the higher-molecular-weight components tended to decrease as the particle size decreased. (As cascade impactor stage number increases, particle size decreases.) The amounts of the components recovered in the solvent trap downstream from the impactor (Table 22) suggest that a significant fraction of the lower-molecular-weight materials was present in the vapor phase.

Analysis of the PS remaining in the generator reservoir after 6 h of aerosol generation showed little change from PS taken directly from stock (Table 23). Therefore, little fractionation of these constituents appears to have occurred during generation of the aerosol.

TABLE 21. Concentrations (ppm) of PNA Compounds on the Stages of a Mercer Cascade Impactor.^(a) The amount of each compound was determined by GC; total mass on each stage was weighed on a microbalance, weight (in μg) of material on each stage is given in parentheses.

Compound	Stage 3 (224)	Stage 4 (448)	Stage 5 (1085)	Stage 6 (1293)	Stage 7 (1436)	Filter (444)	Process Solvent ^(b)
Naphthalene	1,643	1,399	1,449	1,611	1,313	10,391	7,649
2-Methylnaphthalene	11,596	5,254	8,244	8,136	6,331	25,009	35,008
1-Methylnaphthalene	1,636	1,347	1,396	1,404	1,104	2,426	5,489
Biphenyl	4,891	3,871	3,585	2,921	2,340	7,191	9,216
Acenaphthene	5,455	4,593	3,660	3,015	1,911	2,329	9,029
Fluorene	11,597	10,671	9,395	6,314	4,655	4,044	11,935
Phenanthrene	52,663	53,033	44,339	45,947	39,412	11,714	40,683
Fluoranthene	7,935	7,826	9,526	10,846	11,868	5,287	6,209
Pyrene	7,608	5,816	6,750	7,807	8,321	5,816	4,820

(a) The cut-off diameters (μm) for the stages were: Stage 3, 3.61; Stage 4, 2.49; Stage 5, 1.88; Stage 6, 1.23; Stage 7, 0.75 and Filter, 0.33.

(b) For reference and comparison purposes, the concentration of each compound determined in SRC-I process solvent is given in ppm.

TABLE 22. Amounts of PNA Compounds Recovered in a Solvent Trap Downstream from the Mercer Cascade Impactor

<u>Compound</u>	<u>µg Recovered</u>
Naphthalene	78.0
2-Methylnaphthalene	109.9
1-Methylnaphthalene	16.6
Biphenyl	7.1
Acenaphthene	1.9
Fluorene	--- (a)
Phenanthrene	---
Fluoranthene	---
Pyrene	---

(a) Amounts were below detectable limits.

TABLE 23. Comparison of Concentrations of PS Constituents Before and After Aerosol Generation

<u>Compound</u>	<u>Concentration (ppm)</u>	
	<u>before</u>	<u>after</u>
Naphthalene	6,960	6,298
2-Methylnaphthalene	27,611	27,481
1-Methylnaphthalene	5,351	5,324
Biphenyl	10,693	10,809
Acenaphthene	4,595	4,590
Fluorene	8,910	9,100
Phenanthrene	31,424	32,438
Fluoranthene	4,514	4,744
Pyrene	6,792	6,902
1,2-Benzofluorene	1,514	1,480
2,3-Benzofluorene	406	397

A 3-wk experiment was performed in which rats, guinea pigs, mice and shaved mice were exposed 6 h/day for 5 days/wk to one of three concentrations, which averaged 0.60, 0.16 and 0.08 mg/l during the exposure period. Weight changes, general appearance and mortality were recorded. Weight loss was the major effect noted in both male and female rats. Mice also lost weight at both middle and high doses. In addition, all of the female mice in the high-dose chamber died between the second and third weeks of exposure. By 4 weeks after exposure, all rats and mice had regained their lost weight and weighed nearly as much as controls.

4.6 PULMONARY FUNCTION

Since inhalation represents a potential route of exposure to SRC materials, it is of interest to determine whether pulmonary function is altered by exposure to such materials. Most pulmonary function tests are less sensitive in rodents than in larger experimental animals such as dogs. However, data obtained in our laboratory and elsewhere suggest that the response of guinea pig lung to a challenge of inhaled histamine may be a relatively sensitive indicator of pulmonary changes, particularly in small-airway function.

Groups of guinea pigs were therefore exposed to a concentration of 9 µg/l histamine aerosol, after which pulmonary compliance and resistance were determined. Animals were then exposed to 0, 0.16 or 0.60 mg/l of PS aerosol for up to 12 days. All guinea pigs exposed to the high dose died when rechallenged with histamine. Animals exposed to the 0.16 mg/l concentration survived the histamine challenge but exhibited a marked increase in resistance and a marked decrease in compliance relative to the control group (Figures 13a and b). These data suggest that exposure of guinea pigs to relatively high concentrations of PS affect the small airways of the lung. No comparable data for crude petroleums or other synfuel materials are presently available.

4.7 METABOLISM AND DOSIMETRY

Relatively little is known about the biological disposition or the dosimetry of organic chemical mixtures, yet this information is needed in order to

FIGURE 13a. Effect of Process Solvent on Pulmonary Resistance After Histamine Challenge

FIGURE 13b. Effect of Process Solvent on Pulmonary Compliance After Histamine Challenge

relate biological effects to causative agent(s). Moreover, if the toxicological information being developed is to be extrapolated to other species, particularly man, much more detailed knowledge is needed about the rate of absorption, clearance from blood, tissue or organ accumulation, tissue or organ clearance and biological transformation of potential toxicants. In an effort to accumulate such data, we have performed dosimetry studies.

Since our acute, subchronic and developmental toxicity studies involved oral administration of test materials, we began our dosimetric studies by examining the absorption and distribution of selected PS components after administration by gavage. In these studies, PS was administered to Wistar rats and blood samples obtained at times ranging from 15 min to 48 h after administration. Animals were killed from 1 to 48 h postadministration and tissues removed for analysis. Tissues were homogenized and extracted with

pentane and extracts were analyzed by GC. The chromatograms obtained from the extracts were compared with those of PS to determine if the ratios of components in tissue samples were different from those in PS. Since PS contains a very large number of components, only a few major ones were studied initially. Compounds analyzed (percent concentration given in parentheses) were: biphenyl (1.1%), naphthalene (1.0%), 2-methylnaphthalene (4.3%), acenaphthene (0.6%), and phenanthrene (4.9%).

The rate of loss of materials from the stomach contents shows an inverse relationship to the molecular weight (Figure 14). These data also suggest that movement of these materials from the stomach is much slower than is usually found for stomach emptying (a few hours).

The removal of compound recovered from the red blood cells and rate of recovery are shown in Figure 15. Plasma concentrations of all components were much lower than concentrations in red blood cells. Phenanthrene, for example, was present less than 1 $\mu\text{g}/\text{m}$ in plasma at any time point.

FIGURE 14. Half-Life of Compound in Stomach Contents As a Function of Its Molecular Weight

The highest concentrations of biphenyl, 2-methylnaphthalene, and phenanthrene at 4, 7 and 24 h after oral administrations of PS were found in fat (Table 24). Liver contained the next highest concentration of these compounds and the highest concentration of acenaphthene. Kidney had lower concentrations than liver; lungs contained measurable concentrations only of 2-methylnaphthalene and phenanthrene at 7 and 24 h after dosing.

FIGURE 15. Recovery of Compounds from the Red Blood Cells of Rats

TABLE 24. Concentrations of Process Solvent Components in Tissues at Indicated Intervals After Oral Administration

Compound	Concentration (μg/g)										
	Liver			Kidney			Lung		Fat		
	4 hr	7 hr	24 hr	4 hr	7 hr	24 hr	7 hr	24 hr	4 hr	7 hr	24 hr
Biphenyl	1.4	1.3	1.3	0.45	0.50	0.9	--	--	5.0	6.0	15.0
2-methylnaphthalene	2.4	2.4	2.4	1.4	1.5	1.9	0.95	0.95	16.0	19.0	30.5
Acenaphthene	9.0	9.0	14.5	0.41	0.42	1.0	--	--	4.0	4.0	7.1
Phenanthrene	6.0	5.5	5.3	3.3	3.5	6.5	2.5	4.0	29.5	30.0	48.0

5.0 EFFECTS OF SRC-I PRODUCT ON GERMINATION AND GROWTH OF BARLEY

SRC materials may be inadvertently added to soil in transportation spillages or other kinds of accidents that will require recovery and clean-up procedures. The purpose of this investigation is to test the effect of solid SRC-I product added to soil on the growth of barley, a plant often used in biological assay tests.

The SRC-I product, obtained from the pilot plant at Fort Lewis, WA, consisted of black, pea-sized angular pellets that were water-repellent. The experimental soil sample was Ritzville silt loam obtained from the Hanford Site in Benton County, WA. This nonsaline soil has a pH near neutral.

Barley plants were grown outdoors in containers (lysimeters), each consisting of a 1-m-long piece of plastic irrigation pipe, nominally 5 in. inside diameter (13 cm). The lysimeter was placed inside a 6-in.-diameter pipe, which is buried in the ground. Irrigation could be carefully controlled by weighing the lysimeter and adding water to approximate the field capacity of the soil column. This was done as needed throughout the 3-month period required to grow barley from seed to maturation.

The SRC-I material obtained from the pilot plant at Fort Lewis was mixed with soil in two ways. A 3-dm surface layer was superimposed on a 7-dm soil of a 1:1 mixture of soil and SRC-I by dry weight layer, creating a column 1 m deep in the lysimeter. This treatment (mixed) was replicated six times. In six other lysimeters, a 1-dm layer of SRC-I was layered over a 6-dm-deep soil column, and then covered with a 3-dm-deep layer of clean soil. Six other lysimeters were filled with soil alone to serve as controls. In total, 18 lysimeters were used in the test of the solid material: six mixed, six layered and six controls.

There was no evidence that solid material added to the soil produced lesions or chlorotic or necrotic tissues in barley plants. Culms and seed heads were longest on plants grown in control lysimeters and shortest on those grown in soil layered with solid material (Table 25). Barley plants grown in

TABLE 25. Number of Flowering Culms ( ), Length of Culm and Length of Seed Heads of Barley Grown in Soil Treated with SRC-I Material

<u>Treatment</u>	<u>Culm Length (cm)</u>	<u>Seed Head Length (cm)</u>
Control	50 $\pm$ 2.0 (13)	7.4 $\pm$ 2.0
	52 $\pm$ 2.3 (12)	7.3 $\pm$ 0.4
	44 $\pm$ 1.3 (11)	6.8 $\pm$ 0.4
	52 $\pm$ 1.3 (12)	7.0 $\pm$ 0.2
	42 $\pm$ 1.8 (15)	6.5 $\pm$ 0.3
	46 $\pm$ 2.1 (11)	6.8 $\pm$ 0.3
(Average)	48 (12.3)	6.9
Mixed	43 $\pm$ 1.6 (14)	6.4 $\pm$ 0.4
	42 $\pm$ 1.8 (17)	6.1 $\pm$ 0.3
	46 $\pm$ 1.4 (15)	6.9 $\pm$ 0.2
	40 $\pm$ 1.7 (17)	6.1 $\pm$ 0.3
	40 $\pm$ 1.6 (15)	6.2 $\pm$ 0.3
	36 $\pm$ 1.6 (15)	5.4 $\pm$ 0.3
(Average)	41 (15.5)	6.5
Layered	39 $\pm$ 1.4 (9)	5.8 $\pm$ 0.2
	35 $\pm$ 1.7 (10)	5.7 $\pm$ 0.4
	35 $\pm$ 1.9 (12)	5.0 $\pm$ 0.4
	33 $\pm$ 2.3 (7)	5.6 $\pm$ 0.4
	41 $\pm$ 1.1 (8)	6.4 $\pm$ 0.3
	37 $\pm$ 1.4 (8)	5.0 $\pm$ 0.3
(Average)	37 (9.0)	5.6

soil mixed with solid material had culms and seed heads of intermediate length but these plants had more flowering culms than either the control or layered treatments. The amount of dry matter produced in control and mixed treatments were closely similar but the amount of dry matter produced in layered treatments was less (Table 26).

TABLE 26. Dry Weight of Barley (grams) Grown in Outdoor Containers in SRC Solid Materials Mixed with Soil (avg. $\pm$ SE); n = 6

	<u>Control</u>	<u>Mixed</u>	<u>Layered</u>
Straw	8.2 $\pm$ 0.55	8.7 $\pm$ 1.2	4.2 $\pm$ 0.27
Heads	8.8 $\pm$ 0.27	9.4 $\pm$ 0.56	4.9 $\pm$ 0.32
Grain	7.6 $\pm$ 0.21	7.9 $\pm$ 0.44	4.1 $\pm$ 0.27
Total	17.0 $\pm$ 0.80	18.0 $\pm$ 1.0	9.2 $\pm$ 0.56

The low yield of barley grown in layered lysimeters is attributed to the inability of plant roots to penetrate the solid-material layer. This was clearly observed when lysimeters were cut open and the root systems exposed to view.

In our assays, the solid SRC-I material was chemically innocuous to the growth of barley plants.

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