The Mutagenic and Chemical Properties of SRC-I Materials: A Status Report

R. A. Pelroy

January 1981

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THE MUTAGENIC AND CHEMICAL PROPERTIES
OF SRC-I MATERIALS: A Status Report

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SUMMARY

Process solvent (PS) and solid product (dissolved in organic solvent) produced by the Solvent Refined Coal (SRC-I) process at the Wilsonville pilot plant contain mutagenically active components against the Ames-Salmonella strains, Salmonella typhimurium TA98 and TA100. Ames-positive mutagens are apparently concentrated in the moderately polar to strongly polar chemical constituents of both materials. However, the dissolved solid product contains a much higher proportion of very strongly polar mutagens, and may be enriched in acidic--i.e., oxygen-containing mutagens.

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INTRODUCTION

The feasibility of fuel production by direct liquefaction of coal is currently under investigation in the United States. The Solvent Refined Coal Process I (SRC-I), one method of direct coal liquefaction, is at a pilot plant stage of development and is slated to enter the demonstration plant stage. Concomitant with the technological research required in this effort, biomedical and environmental research on the potential impact of SRC-I product process streams and wastes in the workplace and in the environment is also being carried out. Information obtained from this work may eventually aid design engineers in their effort to develop the SRC-I process in an environmentally acceptable fashion.

As part of this effort, SRC-I materials are being tested for their genetic activity in the Ames/Salmonella test system. Results from Ames assay have been shown to correlate with chemical carcinogenesis. The assay is used extensively as a screening test to identify materials for more time consuming and expensive small animal carcinogenesis tests^(1,8) and to correlate chemical composition with biological effect.

The work presented here was done on two SRC-I pilot plant materials: process solvent (PS) and extracts of the solid SRC-I product. The product was extracted both with organic and aqueous solvents, and the extracts assayed for mutagenicity. The PS and organic-solvent solubilized product were also fractionated by high-performance liquid chromatography (HPLC) and the fractions assayed for mutagenicity.

The choice of these two materials for this initial phase of mutagenesis testing of SRC-I materials was determined by two considerations: First, it is known that the high-boiling, high-molecular weight SRC distillates such as PS were mutagenically active in the Ames assay^(2,10,11,12,17). Lower boiling (low-molecular weight) distillate fractions were without genetic activity in the Ames assay. The PS is also a major process stream which gives rise to end products and also recycles through the process. Thus, PS constitutes a material of primary concern relative to work force exposure. The SRC-I solid product will leave the process facility and is, therefore, of both occupational health and environmental concern.

METHODS

Chemical Fractionation

The SRC-I process solvent (PS) and organic solvent extractable chemicals from SRC-I solid product were fractionated by partition chromatography on Sephadex LH20 (Figure 1). The method employed was a modification of a scheme described by Klimisch and Stadler⁽⁷⁾ and further developed for fossil fuels by Jones, Guerin and Clark⁽⁶⁾. Sephadex LH20 was swelled in methanol:water (85:15) and packed in a 2.5 cm x 30 cm glass column. SRC-I samples up to 1.5 g per column run were diluted with hexane and introduced onto the top of the Sephadex column. Chemical constituents in these materials were eluted sequentially with hexane, followed by 10% toluene in hexane, and finally by methanol. Each elution step was carried out with about 300 ml of solvent.

Two different methods were used to add the solid product to the LH20. In the first, 0.5 g of the finely ground solid product was dissolved in toluene/methanol (3/1), added to dry Sephadex and stirred. This mixture was then dried under nitrogen until there was no liquid left. The Sephadex LH20 beads, containing the product, were then added "dry" to the top of a Sephadex column. Alternatively, 0.5 g of the dry ground solid product was added to dry Sephadex and stirred. Hexane was added to the dry mixture with stirring. The swelled material was added to the top of the column. In both cases sequential solution was used: 300 ml hexane, 300 ml toluene/hexane, 900 ml methanol, 900 ml toluene/methanol (3:1), and 300 ml CHCl_3 . Both methods produced equivalent results and Ames data obtained from the second method (i.e., direct addition of product to Sephadex) will be presented.

FIGURE 1. LH20 Fraction Scheme

The Sephadex LH20 method separates essentially according to polarity, with the hexane fraction containing the least polar compounds, and the methanol fraction having compounds of moderate and high polarity. The toluene/hexane fraction is intermediate, comprised of compounds found in both the hexane and the methanol fractions.

Location of various standard compounds in the three solvent fractions is illustrated in Table 1. Polyaromatic hydrocarbon standards (PAH) were eluted in the LH20 hexane fraction. Several azaarene standards were eluted in the toluene/hexane fraction. Standard compounds eluting in the methanol fraction included polyaromatic primary amines (PAA) phenols and various bifunctional aromatic compounds (e.g., azaaromatic amines, diazapolycyclics, hydroxyazaarenes).

The chemical constituents in PS and those extracted with organic solvents from product were also fractionated by high performance liquid chromatography (HPLC) using a semipreparative normal phase amine column with a ternary solvent gradient. Samples thus obtained were dried and redissolved in DMSO for Ames bioassay. The solvent systems used in this separation procedure were as follows:

- hexane, 100% (0-5 min)
- linear gradient of methylene chloride in hexane (5-15 min)
- methylene chloride, 100% (15-20 min)
- linear gradient of isopropanol in methylene chloride (20-30 min)
- isopropanol, 100% (30-35 min).

The approximate elution times of a series of standard compounds are given in Table 2. Organic solvents used in these procedures were of spectral grade quality and were distilled in glass. The deionized water used for extraction of SRC-I product was first passed through a milli-Q system (Millipore) containing two ion exchange resins and two charcoal filters. All glassware was acid cleaned except the columns which were solvent rinsed with sonication.

Mutagenesis Assays

Agar plate mutagenicity assays were performed as described by Ames et al.⁽¹⁾ using four of the Ames-Salmonella test strains: Salmonella

TABLE 1. Standards on Sephadex LH20

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

typhimurium TA98, TA100, TA1535 and TA1537. The mutagen phenotype defining these strains was determined in each experiment as shown in Table 3.

Dimethylsulfoxide (DMSO) was used for most of the work reported. It was used as the solvent for all chemical standard and complex chemical fractions derived from SRC-I. Dried extracts of water samples were redissolved in acetone for bioassay. In addition to the mutagens used to check on the phenotype of the test strains (Table 3), benzo[a]pyrene (Aldrich Co.) was also included as a positive control in every experiment. Negative controls included the

TABLE 2. Standards on NH₂ Column (HPLC)

Nonpolar Region (0-9 min)

Dibenzothiophene

Dibenzofuran

Anthracene

Pyrene

Chrysene

Benzo[e]pyrene

Benzo[a]pyrene

Nitrofluorene

Moderately Polar Region (~9-22 min)

Trimethylaniline

Trimethylpyridine

Quinolines

Acridine

Aminoanthracene

Indole

Aminopyrene

Carbazole

Polar Region (~22-35 min)

Phenol

Naphthol

Hydroxyindole

Hydroxyquinoline

Aminopyridinol

Aminoquinolinol

Ames/Salmonella typhimurium test strains, plus and minus microsomal enzymes (S9), and appropriate solvent controls. Mutagenic response, measured as revertant colonies were counted electronically using a Biotran II automated colony counter (New Brunswick Scientific Co., Inc., Edison, NJ).

TABLE 3. Mutagen Phenotype of Ames Tester Strains used to Bioassay SRC Materials

Compound	$\mu\text{g}/\text{Plate}$	S9	Response for Ames Test Strain:			
			TA98	TA100	TA1537	TA1535
2-Aminoanthracene	1	+	(+)	(+)	(+)	(+)
9-Aminoacridine	50	+	(-)	(-)	(+)	(-)
Nitrosoguanidine	1	-	(-)	(+)	(-)	(+)
Dimethylbenzacridine	10	+	(-)	(+)	(-)	(-)

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

- indicates less than a twofold response above background.

The SRC-I chemical fractions in this study were assayed for mutagenicity at concentrations of 20, 40, 80, 100, 200 and 500 μg , or 2, 4, 8, 10, 20 and 50 μg per petri plate. Each concentration was assayed in duplicate. Stock solutions of the various SRC-I chemical fractions, stored at -80°C when not in use, were prepared at concentrations of 10,000 $\mu\text{g}/\text{ml}$ in DMSO.

Specific mutagenic activity or potency was expressed as revertant colonies of S. typhimurium TA98/ μg SRC-I material. This was estimated by linear regression analysis of dose-response data, i.e., as the slope of the regression line. A positive test was defined as having a maximum mutagenic response in rev TA98/ μg twofold or greater above background, and a correlation coefficient 0.8 or above for the regression line. For quantitative mutagenesis, the Ames experiments were replicated three times, and the average $\pm$ SE of mutagenic potencies was calculated.

Preparation of Liver (S9) Homogenates

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

RESULTS

Ames Assay of LH20 Fractions

Table 4 is a summary of several experiments showing the mutagenic responses of four of the Ames tester strains, (S. typhimurium TA98, TA100, TA1535 and TA1537) to the LH20 fractions of PS.

All fractions were screened with S. typhimurium TA98 (Table 4, column 3). The LH20 hexane fraction from PS was mutagenically inactive against TA98, but activity was detected for the toluene/hexane and methanol LH20 fractions of PS. No mutagenic response was observed without S9 catalyzed metabolic activation (Table 4, column 4).

Because it was negative against S. typhimurium TA98, the LH20 hexane fraction of PS was tested against three other Ames/Salmonella typhimurium strains. These target cells can detect mutagens not detectable by TA98. All of these tests were performed with metabolic activation of the SRC materials. As shown, the hexane fraction was not mutagenic against any of these Ames tester strains (Table 4, columns 5-7). Therefore, either the neutral components from PS which were concentrated in this hexane fraction were not mutagenically active in the Ames test, or their mutagenicity was masked by the complex chemical environment of the hexane fraction.

The crude PS which was positive against TA98 was also assayed against the other Ames tester strains. Only strains TA98 and TA100 were responsive to this material, confirming previous observations with other synthetic fuel mixtures (3,4,10,11,13,14).

Since S. typhimurium TA98 was found to be a better strain than TA100 for quantitative mutagenesis work, it was used to estimate the potency of the crude PS and PS fractions. Results (Table 5) are taken from three replicate experiments using TA98 as the test strain. In the table, the (A) and (B) designations refer to duplicated LH20 fractionations. The mutagenic potencies of similar fractions were reasonably consistent between the (A) and (B) samples. In most cases, the error in these replicate measurements of mutagenic potency was between 10 to 20% of their mean value.

TABLE 4. Ames Mutagenicity for Four Test Strains Versus the PS

LH 20 Fraction	$\mu\text{g Per Plate}$	Mutagenic Response for Ames Tester Strain(a)				
		TA98		TA100	TA1535	TA1537
		+S9	-S9	+S9	+S9	+S9
Hexane	20	118 $\pm$ 12		224 $\pm$ 16	44 $\pm$ 9	15 $\pm$ 1
	40	111 $\pm$ 9		218 $\pm$ 16	26 $\pm$ 2	49 $\pm$ 16
	80	104 $\pm$ 2	ND	234 $\pm$ 18	44 $\pm$ 8	36 $\pm$ 1
	100	78 $\pm$ 10		237 $\pm$ 8	36 $\pm$ 10	34 $\pm$ 12
	200	92 $\pm$ 5		235 $\pm$ 16	34 $\pm$ 12	38 $\pm$ 4
	500	81 $\pm$ 0		185 $\pm$ 10	51 $\pm$ 18	74 $\pm$ 44
Toluene/Hexane	2	78 $\pm$ 1	107 $\pm$ 3			
	4	70 $\pm$ 14	92 $\pm$ 4			
	8	80 $\pm$ 2	122 $\pm$ 4	ND	ND	ND
	10	103 $\pm$ 12	96 $\pm$ 0			
	20	114 $\pm$ 6	94 $\pm$ 8			
	50	150 $\pm$ 8	103 $\pm$ 16			
Methanol	2	168 $\pm$ 16	117 $\pm$ 28			
	4	183 $\pm$ 6	95 $\pm$ 1			
	8	267 $\pm$ 12	66 $\pm$ 0	ND	ND	ND
	10	320 $\pm$ 4	119 $\pm$ 11			
	20	472 $\pm$ 83	119 $\pm$ 11			
	50	676 $\pm$ 64	90 $\pm$ 8			
Crude Process Solvent (unfractionated)	20	181 $\pm$ 7		202 $\pm$ 14	146 $\pm$ 50	72 $\pm$ 5
	40	260 $\pm$ 6		272 $\pm$ 18	82 $\pm$ 11	107 $\pm$ 3
	80	280 $\pm$ 4	ND	262 $\pm$ 10	101 $\pm$ 0	89 $\pm$ 4
	100	296 $\pm$ 39		294 $\pm$ 18	98 $\pm$ 10	110 $\pm$ 10
	200	358 $\pm$ 4		314 $\pm$ 16	77 $\pm$ 8	76 $\pm$ 2
	500	434 $\pm$ 19		312 $\pm$ 10	---	108 $\pm$ 18
Negative Solvent Controls:		109 $\pm$ 14 ^(b)		225 $\pm$ 0 ^(e)		
		67 $\pm$ 14 ^(c)		142 $\pm$ 14 ^(f)	54 $\pm$ 2 ^(g)	54 $\pm$ 2 ⁽ⁱ⁾
		118 $\pm$ 7 ^(d)			112 $\pm$ 0 ^(h)	102 $\pm$ 6 ^(j)

(a) Revertants per plate $\pm$ SE, duplicate samples.

(b) Correspond to methanol fraction, TA98.

(c) Corresponds to toluene/hexane fraction, TA98.

(d) Corresponds to crude process solvent, TA98.

(e) Corresponds to hexane fraction, TA100.

(f) Corresponds to crude process solvent, TA100.

(g) Corresponds to hexane fraction, TA1535.

(h) Corresponds to crude process solvent, TA1535.

(i) Corresponds to hexane fraction, TA1537.

(j) Corresponds to crude process solvent, TA1535.

ND Not Determined..

TABLE 5. Summary of Ames Bioassay Data for LH20 Sephadex Fractions from SRC-I Process Solvent

LH 20 Fraction	Conc. Range ($\mu\text{g}/\text{plate}$)	Rev TA98/ $\mu\text{g} \pm \text{SE}$ (per plate)	wt%	Weighted Activity ^(a)
Hexane (A)	20 to 500	0	75.0	0
Hexane (B)	70 to 500	0	74.0	0
Toluene/Hexane (A)	2 to 50	5.87 ± 0.09	7.1	0.42
Toluene/Hexane (B)	2 to 50	4.27 ± 2.01	8.2	0.35
Methanol (A)	2 to 50	18.31 ± 1.88	16.5	3.02
Methanol (B)	2 to 50	24.98 ± 1.06	15.4	3.85
Crude (unfractionated)	20 to 500	0.54 ± 0.07	100	0.54

$$\text{Sum (A) Fractions} = 3.44; \text{ recovery (A)} = \frac{3.44}{0.54} \times 100 = 637\%$$

$$\text{Sum (B) Fractions} = 4.20; \text{ recovery (B)} = \frac{4.20}{0.54} \times 100 = 778\%$$

(A) and (B) refer to duplicate fractionations of process solvent by LH 20 Sephadex.

(a) Product of wt% times specific mutagenic activity in rev TA98/ μg .

Methanol fractions contained most of the mutagenic activity recovered from PS; i.e., 88% from fractionation (A) and 92% from (B). The weight percent of the LH20 fractions (Table 5, column 4) multiplied times their specific mutagenic activities (Table 5, column 3) yielded a weighted specific mutagenic activity (column 5) which relates the contribution of a given LH20 fraction to the whole PS. These weighted activities, when summed, indicate the extent to which mutagenicities in given chemical fractions are additive (i.e., independent of composition).

The sum of mutagenicities in the individual LH20 fractions was greater for weighted specific mutagenic activity than the measured mutagenic activity

of the crude unfractionated PS. These data suggest a large degree of interference in the expression of Ames mutagenicity in the crude PS that is removed by chemical fractionation by the LH20 procedure.

Tables 6 and 7 summarize Ames mutagenicity data for SRC-I solid product that had been completely solubilized in a series of organic solvents (see Methods). Qualitatively, the mutagenic responses of the solubilized product (Table 6) were the same as those obtained for PS. For example, both S. typhimurium TA98 and TA100 were mutagenized by the toluene/hexane and methanol LH20 fractions, and the crude (unfractionated), solubilized product (Table 6; columns 3 and 4). All mutagenic activity was indirect (Table 6, column 3 versus 4). Assay of crude product showed little or no mutagenic activity using Ames tester strains TA1535 or TA1537. The hexane fraction did not show activity against any of the Ames tester strains used in these assays.

In contrast to results obtained for PS, LH20 chromatography of product required use of two additional solvents to remove highly polar compounds from the Sephadex columns. One of these, a chloroform fraction, was mutagenically active in the Ames test. The second, a toluene/methanol fraction, was mutagenically inactive against the four Ames tester strains.

Table 7 shows mutagenic potency for each of the LH20 fractions and for the unfractionated product. As for PS, potencies (or mutagenic specific activities) represent the mean $\pm$ SE from three replicate experiments. The two fractionations, labeled (A) and (B), were again compared to check on the mutagenic consistency of the same fractions obtained at different times (i.e., in duplicate LH20 fractionations).

Reproducibility of Ames data for any given mutagenic fraction was generally within 20% of its mean specific mutagenic activity value. However, more variation than this was seen between the same fractions from replicate LH20 runs--i.e., (A)'s versus (B)'s. Both (A) and (B) methanol fractions were more active than the other active LH20 fractions (i.e., toluene/hexane or chloroform fractions). However, the specific mutagenic activities of the toluene/hexane and chloroform fractions varied so much between the (A) and (B) runs that it was impossible to assign a difference between these fractions in terms of their mutagenic specific activity.

TABLE 6. Ames Mutagenicity Data for Four Test Strains Versus the Organic Fraction from Product

LH 20 Fraction	$\mu\text{g Per Plate}$	Mutagenic Response for Ames Tester Strain(a)				
		TA98		TA100	TA1535	TA1537
		+S9	-S9	+S9	+S9	+S9
Hexane	20	145 $\pm$ 6		182 $\pm$ 2	48 $\pm$ 11	35 $\pm$ 1
	40	172 $\pm$ 13		201 $\pm$ 9	35 $\pm$ 1	56 $\pm$ 22
	80	187 $\pm$ 17	ND	230 $\pm$ 8	48 $\pm$ 2	57 $\pm$ 10
	100	174 $\pm$ 20		244 $\pm$ 10	48 $\pm$ 14	56 $\pm$ 4
	200	236 $\pm$ 8		264 $\pm$ 15	90 $\pm$ 12	88 $\pm$ 1
	500	231 $\pm$ 40		---	---	28 $\pm$ 4
Toluene/Hexane	2	146 $\pm$ 16	114 $\pm$ 20			
	4	174 $\pm$ 9	96 $\pm$ 5			
	8	248 $\pm$ 10	74 $\pm$ 1	ND	ND	ND
	10	300 $\pm$ 40	101 $\pm$ 15			
	20	407 $\pm$ 17	92 $\pm$ 2			
	50	566 $\pm$ 48	114 $\pm$ 10			
Methanol	2	142 $\pm$ 4	136 $\pm$ 20			
	4	217 $\pm$ 9	108 $\pm$ 20			
	8	338 $\pm$ 52	110 $\pm$ 6	ND	ND	ND
	10	434 $\pm$ 48	100 $\pm$ 4			
	20	720 $\pm$ 84	76 $\pm$ 4			
	50	1201 $\pm$ 92	91 $\pm$ 7			
Chloroform	2	112 $\pm$ 10				
	4	137 $\pm$ 2				
	8	172 $\pm$ 13	ND	ND	ND	ND
	10	204 $\pm$ 5				
	20	264 $\pm$ 16				
	50	526 $\pm$ 38				
Toluene/Methanol	20	123 $\pm$ 4		182 $\pm$ 2	89 $\pm$ 4	78 $\pm$ 5
	40	110 $\pm$ 4		201 $\pm$ 9	90 $\pm$ 8	104 $\pm$ 30
	80	129 $\pm$ 1	ND	230 $\pm$ 8	111 $\pm$ 3	112 $\pm$ 3
	100	113 $\pm$ 8		244 $\pm$ 10	124 $\pm$ 10	104 $\pm$ 12
	200	121 $\pm$ 3		264 $\pm$ 15	109 $\pm$ 19	152 $\pm$ 6
	500	106 $\pm$ 1		---	---	---
Crude Product (unfractionated)	20	154 $\pm$ 73		176 $\pm$ 10	146 $\pm$ 50	74 $\pm$ 13
	40	350 $\pm$ 50		254 $\pm$ 28	82 $\pm$ 11	140 $\pm$ 10
	80	596 $\pm$ 20		284 $\pm$ 12	101 $\pm$ 0	102 $\pm$ 1
	100	638 $\pm$ 16		307 $\pm$ 15	98 $\pm$ 10	86 $\pm$ 8
	200	896 $\pm$ 62		390 $\pm$ 53	170 $\pm$ 3	107 $\pm$ 38
	500	1106 $\pm$ 14		1198 $\pm$ 29	---	
Negative Solvent Control	---	109 $\pm$ 14	125 $\pm$ 0	230 $\pm$ 8	50 $\pm$ 5 112 $\pm$ 3(b)	54 $\pm$ 2 102 $\pm$ 6(c)

(a) Revertants per plate $\pm$ SE, duplicate samples.

(b) Corresponds to toluene/methanol fraction and crude product versus TA1535.

(c) Corresponds to toluene/methanol fraction and crude product versus TA1537.

ND Not Determined.

TABLE 7. Summary of Ames Bioassay Data for LH20 Sephadex Fractions from SRC-I Product Solubilized in Organic Solvents

<u>LH20 Fraction</u>	<u>Conc. Range ($\mu\text{g}/\text{plate}$)</u>	<u>Rev TA98/μg $\pm\text{SE}$</u>	<u>wt%</u>	<u>Weighted Activity^(a)</u>
Hexane (A)	20 to 500	0	31.9	0
Hexane (B)	20 to 500	0	25.2	0
Toluene/Hexane (A)	2 to 50	17.25 ± 2.98	7.7	1.33
Toluene/Hexane (B)	2 to 50	7.58 ± 1.54	12.7	0.96
Methanol (A)	2 to 50	32.25 ± 1.04	16.2	5.22
Methanol (B)	2 to 50	40.25 ± 3.84	18.3	7.37
Chloroform (A)	2 to 50	9.68 ± 1.12	4.2	0.41
Chloroform (B)	2 to 50	13.05 ± 2.22	10.7	1.40
Toluene/Methanol (A)	20 to 500	0	38.4	0
Toluene/Methanol (B)	20 to 500	0	44.5	0
Crude Unfractionated Extract	20 to 500	7.02 ± 0.57	100	7.02

Sum (A) Fractions = 6.96 rev/ μg ; recovery, $\frac{6.96}{7.02} \times 100 = 99\%$

Sum (B) Fractions = 9.73 rev/ μg ; recovery, $\frac{9.93}{7.02} \times 100 = 139\%$

(A) and (B): See Table 2.

(a) See Table 2.

Weighted specific mutagenic activities (Table 7, column 5) again showed that most of the mutagenicity recovered from the product by LH20 chromatography was found in the methanol fractions [i.e., 75% for (A) and 74% for (B)]. Compared to the unfractionated (solubilized) product, recoveries of mutagenicity in the LH20 fractions ranged from 99% for (A) to 139% for (B). These data suggest that interference with mutagenicity measurements by Ames assay was not as great for the unfractionated, solubilized product as it was for the unfractionated PS.

Ames Assay of HPLC Fractions

The HPLC separation of components in PS and SRC-I solid product revealed quantitative differences in the mutagens in these two SRC-I complex mixtures (Figures 2 and 3). Mutagenicity of PS (Figure 2) was associated mainly (i.e., ca 80% of recovered mutagens) with moderately polar chemical constituents that elute with retention times similar to nitrogen heterocycles and primary aromatic amines (PAA). A smaller mutagenic component eluted in an HPLC region characterized by strongly polar constituents; e.g., phenols, mixed nitrogen compounds containing phenolic groups.

Mutagenic activity of product (Figure 3) was more diffuse with the main component of activity (ca 58%) in the strongly polar LC region, and somewhat less (ca 35%) in the moderately polar to strongly polar regions, and the smallest amount of mutagenic activity in the moderately polar region (ca 13%).

FIGURE 2. Ames Mutagenic Activity of LC Fractions of PS. Top panel: absorbance of LC fractions at 250 nm. Bottom panel: total mutagenic response in revertants TA98 (specific mutagenic activity in rev TA98/μg x μg fraction) and specific mutagenic activity.

FIGURE 3. Ames Mutagenic Activity of LC Fractions of the Organic Soluble Fraction of Product. Top panel: absorbance of LC fractions at 250 nm. Bottom panel: total mutagenic response in revertants TA98 (specific mutagenic activity in rev TA98/μg x μg fraction); and specific mutagenic activity.

DISCUSSION

Like other coal and oil shale synthetic fuels^(3,4,10,11,13,14), the high-boiling SRC-I solid product and PS dissolved in organic solvent contained mutagens detectable in the Ames test. These mutagens were concentrated mainly in chemical fractions enriched in polar and moderately polar constituents and, in particular, in nitrogen-containing polycyclics.

As hydrocarbon mixtures, SRC high-boiling materials appear to be relatively rich in azaarenes^(5,9,16), neutral polyaromatic compounds⁽¹⁶⁾ and may also contain PAA^(12,17). Both azaarenes and PAA's would have been concentrated in the most mutagenically active chemical fractions from PS and in some of the less active fractions from product. The chemical characterization of both product and PS is now underway.

Also in common with previously reported results^(3,4,11,13,14), Ames mutagenicity, either of the crude SRC-I materials or their chemical fractions, was indirect and required a source of metabolic activation of the material. S. typhimurium TA98 was the most sensitive of four tester strains to mutagenesis induced by SRC-I product and PS. Thus, it was used for estimating the mutagenic potency of these SRC-I materials. Other synfuels, including coal-derived jet fuels, were also shown to contain mutagens highly effective against TA98^(3,4,11,13,14).

In relative terms, PS and the organic solvent solubilized product were of approximately the same mutagenic potency as coal-derived jet fuels⁽⁴⁾ and SRC-II high-boiling range distillates^(10,12). They were also somewhat more genetically active in the Ames assay than shale oils and shale oil fractions^(10,11,13).

The PS and product differed in the way mutagenic activities distributed in LH20 and HPLC chemical fractions. This took the form of additional LH20 fractions required to recover all mutagenic activity for product, but was especially evident for the HPLC fractions of product. For example, in the PS, like other high-boiling range SRC liquids, mutagenically active constituents were concentrated in HPLC fractions identified with moderately polar nitrogen

heterocyclics and PAA. On the other hand, more than half of the mutagenic activity recovered after chromatography of the product was localized in fractions identified with highly polar acidic constituents (e.g., phenolic compounds).

The Ames dose-response curves for unfractionated PS were nonlinear and yielded lower estimates of mutagenic potency than those obtained by summation of the weighted potencies of the PS chemical fractions. Conversely, the unfractionated (organic solvent solubilized) product had about the specific mutagenic activity of the sum of the chemical fractions from this material weighted for percent composition in the crude.

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