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BIOLOGICAL MONITORING OF OIL SHALE PRODUCTS AND EFFLUENTS USING SHORT-TERM GENETIC ANALYSES

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ABSTRACT

The long-term health hazards such as mutagenesis, carcinogenesis and teratogenesis due to the exposure to crude shale oil, particulate pollutants and the leachates from raw or spent shale constitute a major concern in the development of shale oil technology. In order to monitor such biological effects, we have applied short-term genetic analyses with the exemplary test materials. The Salmonella/microsomal activation system (Ames assay) was generally applicable but only upon chemical fractionation. The Stedman liquid-liquid extraction procedure or the Sephadex gel filtration (LH-20) technique were effectively utilized. Mutagenicity analyses with various crude oils and product water have revealed biological activity in the basic (aromatic amine fractions) or in the neutral (polyaromatic hydrocarbon fraction) fractions. Extracts and chromatographically isolated materials from raw and spent shale were subjected to mutagenicity studies. Mutagenic activity was noted and correlates with the biological activity of compounds that are either identified or predicted to occur in these materials. Comparison to other energy technologies and overall health hazard of the test materials will be discussed.

INTRODUCTION

The long term health hazards such as toxicity, mutagenicity, carcinogenicity and teratogenicity are of great concern in the development of new alternate energy technologies, including the oil shale industry. Exposure of the personnel in industry as well as consumers to the oil shale deposits, contaminated aqueous effluents and air born particulates might constitute the biological hazard. In addition the entry of leachates from raw and spent shale in to drinking water systems represents another potential route of entry into human environment. Thus, the need for biological monitoring of such processes is obvious and every effort should be made to minimize the toxic and genotoxic effects associated with this industry.

The approach for biological monitoring of the shale oil industry is two fold. (1) Development of an adequate biological testing (quality control) to monitor various processes, effluents or personnel in the development of the engineering and control technology. Various genetic-toxicological test procedures are now available for the detection and isolation of biological hazard. However, it is necessary to identify which of these procedures can be advantageously, appropriately and economically applied (quality control) in determining biological effects. (2) Once after the base line biological data is developed, it is necessary to periodically monitor the processes when they are completely developed for full size commercial production.

In order to rapidly and inexpensively ascertain the potential mutagenicity hazards of various test materials, we have examined the feasibility of using short-term genetic assays to predict and, in some cases, aid in isolating and identifying chemical mutagens. Furthermore, recent studies [1] have shown that there is an extremely high correlation

between the ability of a compound to induce genetic damage and the carcinogenic potential of the compound. Thus, the mutagenicity assay might act as a prescreen for carcinogens. In the studies presented here we have used the Ames Salmonella histidine reversion assay [1] to assay the mutagenic potential of chemically fractionated [2] crude shale oil, product water from shale oil process [3] and chromatographically separated leachates and extracts from raw and spent shale [4].

In order to maintain the uniformity of samples that are tested at various laboratories, the repository [5] at the Oak Ridge National Laboratory, Oak Ridge (supported by the U.S. Environmental Protection Agency) collects and supplies adequate amounts of exemplary materials from the oil shale and other related energy technologies. Materials used in this study were obtained from the ORNL repository and Dr. J. J. Schmidt-Collerus, Denver Research Institute, Denver, Colorado.

MATERIALS AND METHODS

A. Mutagenicity Testing - Methodology

The Salmonella typhimurium strains used in various assays are listed below. All were obtained through the courtesy of Dr. Bruce Ames, Berkeley, California.

TA100 hisG46, uvrB, rfa (missense plus R factor)

TA98 hisD3052, uvrB, rfa (frameshift plus R factor)

In the screening of fractionated materials, the two strains TA98 and TA100 were generally employed. Standard experimental procedures have been given by Ames et al. [1]. Briefly, the strain to be treated with the potential mutagen(s) is added to soft agar containing a low level of histidine and biotin along with varying amounts of the test substances.

The suspension containing approximately 2 X 10^8 bacteria is overlaid on minimal agar plates. The bacteria undergo several divisions with the reduced level of histidine, thus forming a light lawn of background growth on the plate and allowing the mutagen to act. Revertants to the wild-type state appear as obvious large colonies on the plate. The assay can be quantitated with respect to dose (added amount) of mutagen and modified to include "on-the-plate" treatment with the liver homogenate required to metabolically activate many compounds.

Fractions and/or control compounds to be tested were suspended in dimethyl sulfoxide (supplied sterile, spectrophotometric grade from Schwarz/Mann) to concentrations in the range of 10-50 mg/ml solids. Normally, the fraction was tested with the plate assay over at least a 1000-fold concentration range with the two tester strains TA98 and TA100. Revertant colonies were counted after 48 h incubation. Data were recorded and plotted vs added concentration, and the slope of the induction curve was determined (Figure 1). It is assumed that the slope of the linear dose-response range reflects the mutagenic activity. Metabolic activation for pro-carcinogens was incorporated into the assay by the addition of rat liver microsomal enzymes (liver S-9 mix from rats induced with Aroclor). Routine controls demonstrating the sterility of samples, enzyme or rat liver S-9 preparations, and reagents were included. Positive controls with known mutagens were also included in order to recheck strain response and enzyme preparations. All solvents used were non-mutagenic in the bacterial test system.

B. Samples - Source

(1) A crude shale-oil sample from the above-ground simulated in situ oil-shale retorting process; (2) the aqueous product water consisting of the centrifuged water of combustion from the same process (both samples 2 and 3 courtesy of Dr. Richard Poulson of the Laramie Energy Research Center). (3) Carbonaceous spent shale from the TOSCO Process was obtained by the courtesy and cooperation of Colony Corporation (ARCO) and the second from the Paraho (Direct Mode) Pilot processing plant[a]. (4) A 382 g sample of unretorted shale in the form of air particulate was collected by High Vol collector at the Paraho site[a].

C. Chemical Fractionation

(1) Class fractionation scheme: The fractionation technique developed by Swain et al. [2] and modified by Bell et al. [6] was used to fractionate the oil samples and the aqueous samples. The technique involves acid-base separation using liquid-liquid partioning. The neutral fraction was fractionated into secondary fractions using ~~fluoracil~~ Florisil column and elution with hexane, benzene, ether and methanol. The acidic and basic fractions were separated into ether or water soluble secondary fractions.

(2) Since acid-base separation technique involves harsh chemical treatment, a much gentler technique developed by Jones et al. [7] using Sephadex LH-20 gel filtration technique was used. The technique utilizes the separation of hydrophilic and lipophilic fractions in Step I, separation of polymeric, seived and hydrogen-bonding fraction from lipophilics in the Step II and finally separation of aliphatic and aromatic fractions (ring size) from seived fraction in the Step III.

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[a]Obtained by Dr. J. J. Schmidt-Collerus.

(3) Extraction of raw and spent shale: Each shale sample was Soxhlet extracted for 6 days with benzene and the residue of benzene solubles was then concentrated by distilling off the solvent [4]. The final concentration of the benzene solubles lie in the range of 10-20 mg/ml (a range suitable for TLC separation). Chromatographic separation of the complex benzene extract into polynuclear aromatic hydrocarbons (PAH, neutral), polar compounds (azaarenes, phenols, etc.) and other non-polar hydrocarbons was achieved by using one-dimensional silica gel thin layer chromatography and was described previously [4]. The saturated hydrocarbons run with the solvent at the top of the plate; PAH's run in a group forming a wide fluorescing band in the top portion of the plate. Polar compounds migrate through the lower half of the plate separating into bands while most polar materials remain at the origin. The PAH residue was subjected to a second separation on a silica gel plate to achieve a higher quality of separation. Separation of the individual PAH compounds was achieved with reasonable success on a two-dimensional mixed thin-layer chromatographic plate.

RESULTS AND DISCUSSION

A. Oil Samples

In the investigation of the feasibility of the coupled analytical-mutagenicity assay approach, we examined the mutagenic activity of fractionated in situ retorted shale-oil sample (simulated). Each primary fraction was assayed with the Ames strains. The distribution by weight of the test materials, the "specific activity" (revertants/mg) of each fraction, and the contribution of each fraction to the mutagenic potential of the starting material (product of weight percent and specific activity)

are listed in Table 1. Data are given for the frameshift strain TA98 with metabolic activation with enzyme preparations from Aroclor 1254-induced rats. The shale-oil contained significant activity in the neutral fractions and in other fractions, particularly in the Basic Fraction (B_E, ether soluble). Note that the sum of activities from the neutral subfractions corresponds to the value obtained from the unfractionated neutral material.

Figure 1 shows the dose-response curves for two of the shale-oil fractions. The slope of the linear portion of the induction curve represents the revertants/mg of the fraction (specific activity).

Comparable evaluations of crude synthetic fuels from coal liquefaction processes have pointed to consistently higher mutagenic potentials in synthetic fuels than in the materials assayed here [8].

B. Aqueous Sample

In order to extend the techniques to an aqueous material that might have more environmental importance, we assayed the centrifuged product water from the above-ground in situ retorting process (Table 2). Although a number of highly active materials occur, again in the basic fractions, the overall contribution of the contaminating organic portion appears to be low. Note also that the neutral portion, usually comprised of water-insoluble polyaromatic hydrocarbons, contains little mutagenic activity in this aqueous sample.

Since the LH-20 gel filteration technique is a much gentler system, we have examined the feasibility of testing shale oil samples fractionated with LH-20 fractionation scheme. The results are given in Table 3 which shows the general applicability of this technique for biological testing. The activity was recoverable completely (see summation column, Table 3). Total mutagenic activity recovered after fractionation (280 rev/mg) is comparable to the activity obtained with the acid-base fractionation technique (178 rev/mg).

Table 4 lists the results of mutagenicity testing (strain TA98 and TA100, with metabolic activation) with the extracted and chromatographically separated materials from Tosco II series (CSA II respresents test samples from the Tosco process spent shale). The first sample, Diffuse fluorescent material combined (DF COM) was derived from a benzene extract. The sample represents the combined material from 5 TLC plates and is, in general, similar to the neutral or Polycyclic aromatic hydrocarbon fraction from acid-base fractionation scheme. The main constituents are probably a homologous series of alkyl substituted and poly-condensed substituted aromatic hydrocarbons. The mutagenicity testing results detect mutagenic activity.

The next samples represent the polar material, from Tosco II spent shale. The recovered materials are roughly analogous to a basic fraction by the acid-base extraction technique. Predictions would include nitrogenous polycondensed species, acridine, dibenzacridines, along with some acids, phenols and high molecular weight aromatic amines. Mutagenicity can be detected with the Salmonella system. Note in Table 2 that samples D-1135 and D-1165 differ quantitatively and qualitatively. Conceivably, minor changes in the extraction procedures and chromatography can alter the bioassay results.

When the total benzene soluble fraction from Paraho spent shale is analyzed, toxicity masks any mutagenic effect that might be present. However, a similar crude extract from raw shale (APVI [1]) air particulate was assayable and mutagenic activity was detectable.

C. Utility of Short-Term Tests for Mutagenicity

The use of short-term tests for mutagenicity coupled with chemical fractionation and analyses of test materials appears to be a valid research approach. Their utility in predicting potential genetic hazard

is obvious. The use of the mutagenicity data as a prescreen for carcinogenesis may also be of value, but probably not in a quantitative sense. Too many factors modify the whole-animal carcinogenesis response to expect the type of mutagenicity screening used here to directly reflect the extent of carcinogenic potential.

The biological testing is a complex phenomenon which warrants extreme caution in its application and interpretation. Implication of various genetic and biochemical variables was previously described [9]. The choice of bacterial strain or the inducer involved in metabolic activation could alter the test results. Furthermore, no one short-term test should be relied on for testing. Other systems [10] might complement one another.

However, in the context of a prescreen for mutagenesis, and perhaps for carcinogenesis, the testing of crude mixtures with the Ames system is a feasible approach provided that appropriate fractionation, chemical analyses, and validation accompany the bioassays. A more important use of the short-term mutagenicity tests may lie in the dissection of a known response in a crude material and the tracing of the effect to the ultimate organic component(s) responsible for the potential damage. The need exists for standardizing test procedures so that they can be routinely utilized for biological monitoring of processes and process streams associated with oil shale technology.

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TABLE 1

DISTRIBUTION OF MUTAGENIC ACTIVITY IN FRACTIONS OF SHALE OILS[a]

Fraction[b]		Shale Oil		
		Relative weight, % of total	Specific activity, rev/mg[c]	Weighted activity, rev/mg[d]
1. $NaOH_I$		1.02	256	3
2. WA_I		0.05	185	>1
3. WA_E		1.23	52	1
4. SA_I		0.09	0	--
5. SA_E		0.26	159	>1
6. SA_W		0.55	160	1
7. B_{Ia}		0.20	1377	3
8. B_{Ib}		0.26	800	2
9. B_E		7.11	952	68
10. B_W		0.28	223	1
11. Neutral		86.66	112 (109)[e]	97
TOTAL		97.71		178
Neutral subfractions				
Hexane	A	58.69	40	23
	B	2.14	625	13
	C	1.27	750	10
Hexane/benzene	A	4.38	238	10
	B	1.89	340	6
	C	1.39	320	4
Benzene/ether	A	12.43	65	8
	B	2.19	142	3
	C	1.29	253	3
Methanol	A	15.12	179	27
	B	0.49	684	3
	C	0.93	263	2
SUBTOTAL		102.21		112

[a]All assays carried out in the presence of crude liver S-9 from rats induced with Aroclor 1254.

[b]I = insoluble (fractions a and b), E = ether soluble, W = water soluble, WA = weak acid, SA = strong acid, and B = base.

[c]rev/mg = revertants/milligram (strain TA98). Values are derived from the slope of the induction curve.

[d]Weighted activity of each fraction relative to the starting material is the product of columns one and two. The sum of these products is given as a measure of the total mutagenic potential of each material. The value for the neutral fraction was calculated from the value for the weighted subfractions.

[e]Activity based on assay of the total neutral fraction before chromatography rather than on the summation of the individual subfraction.

TABLE 2

DISTRIBUTION OF MUTAGENIC ACTIVITY IN FRACTIONS OF AQUEOUS SAMPLE[a]

	Fraction[b]	Shale-oil product water		
		Relative weight[c] % of total	Specific activity, rev/mg	Weighted activity, rev/mg
1.	$NaOH_I$	--	--	--
2.	WA_I	1.5	397	5
3.	WA_E	6.3	105	7
4.	SA_I	3.9	0	--
5.	SA_E	16.8	0	--
6.	SA_W	65.0	0	--
7.	B_{Ia}	0.1	52	<1
8.	B_{Ib}	0.1	1468	1
9.	B_E	2.7	1575	42
10.	B_W	1.3	868	12
11.	Neutral	2.4	52	1
	Total			68

[a]See footnotes to Table 1.

TABLE 3

MUTAGENIC ACTIVITY OF SHALE OIL FRACTIONED WITH SEPHADEX LH-20 SYSTEM

		Specific Activity[a] (Rev/mg)	Weighted Activity	Summation of Fractions
1.	Original	750	750	
3.	Hexane Insol.	1750	23	413
4.	Hexane sol.	400	390	
5.	Hydrophilic	1400	103	248
6.	Lipophilic	175	145	
7.	Polymeric	250	6	
8.	Solved	200	155	200
9.	H-Bonded	750	39	
10.	Aliphatic	0	0	
11.	Mono-aromatic	78	4	
12.	Di & Tri-aromatic	800	49	109
13.	Poly-aromatic	2800	56	
	TOTAL		280[c]	

[a]Results obtained with strain TA98 and metabolic activation with Aroclor induced rat liver S-9 mix.

[b]Refer to Table 1.

[c]Summation of fractions 3, 5, 7, 9, 10-13.

TABLE 4

MUTAGENICITY RAW AND SPENT SHALE

Sample Designation	Fraction Tested	Mutagenicity his^+ REV/mg[a]	
		TA98	TA100
CSA II (1) (TOSCO)	Diffused Fluorescence	220	360
CSA II (1) (TOSCO)	Polar D113S Material D116S	500 320	400 0
CSA II (2) (TOSCO)	Polar D97S Material	220	0
SA VII (1) (PARAHO)	Total Benzene Soluble	Toxic	Toxic
AP VI (1) (PARAHO)	Total Benzene Soluble	60	440

[a]Slope of dose-response curve.

FIGURE LEGEND

Figure 1. Induction of revertants in Salmonella strain TA98 with increasing concentration of (A) Fraction 9, basic, ether-soluble from shale oil; and (B) Fraction 9, basic, ether-soluble from product water with activation with an enzyme (S-9) prepared from rat livers induced with Aroclor 1254.

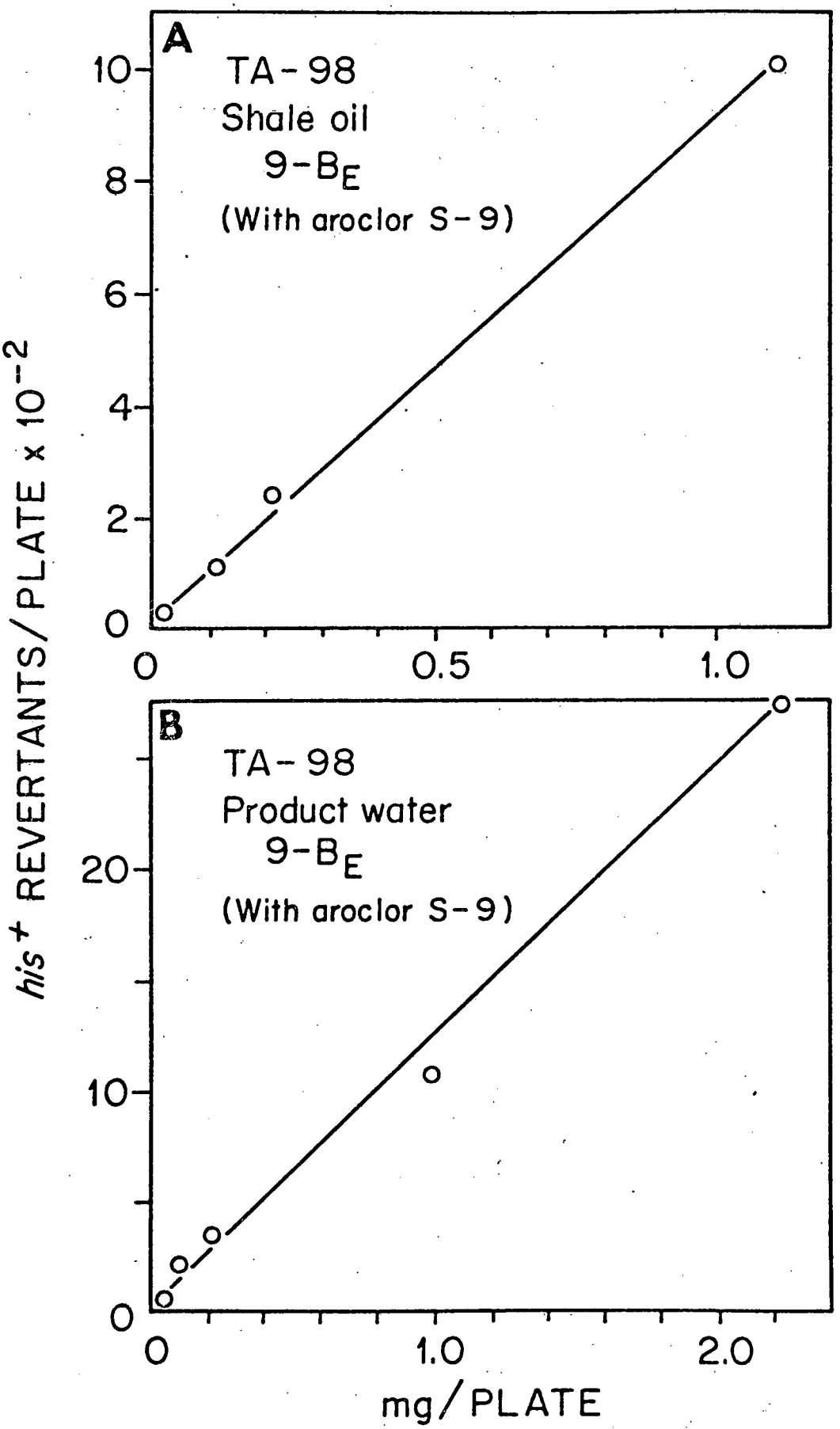
A
TA-98
Shale oil
9-B_E
(With aroclor S-9)
B
TA-98
Product water
9-B_E
(With aroclor S-9)
his+ REVERTANTS/PLATE x 10^{-2}
mg/PLATE
0
2
4
6
8
10
20
0.5
1.0
2.0

Fig. 1