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MUTAGENICITY OF FRACTIONATED TEST MATERIAL FROM THE SYNTHETIC FUEL TECHNOLOGY WITH BACTERIAL SYSTEMS[1]

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DISCLAIMER

SUMMARY

The predictive value of short-term genetic tests, such as the Salmonella and Escherichia coli (K-12, 343/113) systems including microsomal activation, is well documented. We have applied the short-term testing to various crude products and effluents from the synthetic fuel technologies. Class fractionation and column chromatography of the test materials and the coupled bioassays can be used to identify the most active fractions (collaborative effort with Analytical Chemistry Division). Reversion at the histidine locus for Salmonella was assayed with each fraction and the results are expressed in units of revertants (strain TA98) per milligram of the starting material (organic content) including metabolic activation with a crude rat liver preparation. Results obtained with the Salmonella system were validated by employing E. coli strains auxotrophic for arginine. Genetic activity is seen with a variety of fractions, largely the basic and neutral (PAH) components. Total activity varies from process to process, thus, the short-term genetic test can be considered a useful prescreen for potential biohazard of various effluents both in plants and in the immediate plant environment.

INTRODUCTION

The enormous amount of industrial and technological activity in the modern world creates a large number of chemical pollutants. The developing synthetic fuel industry is only one example that could have significant environmental impact on man. The exposures of workers to crude synthetic oils, contaminated aqueous materials, particulate matter, and air pollutants represent potential hazards. Adverse human health effects might result, particularly from long-term exposure to polycyclic aromatic hydrocarbons and trace elements emitted in various forms from the synthetic fuel industry.

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 system [2] and G. Mohn's E. coli (343/113) strain [3] to assay the the mutagenic potential of natural crude oil, synthetic fuels from coal liquefaction and crude shale oil. Mutagenicity data on isolated or suspected organic components are also presented. The results support the use of the short-term genetic tests in examining crude mixtures and point to the advantages of coupling the bioassays with chemical fractionation.

MATERIALS AND METHODS

Samples. Samples which have been surveyed and their sources are: (1) coal-liquefaction products, from the COED Pyrolysis Process, Courtesy of FMC; (2) a crude shale-oil sample from the above-ground simulated in situ oil-shale retorting process; and (3) a natural crude oil (control) from the Larimie Energy Research Center. The authors recognize the possibility that these samples may bear no relationship to the process as it may exist in the future nor should it be construed that these materials are representative of all synthetic crude oils. They are used here simply as appropriate and available materials for the comparative study.

Fractionation. The fractionation scheme described by Swain et al [7] as modified by Bell et al. [8], has been applied to products from coal-liquefaction processes and natural crudes. The separation scheme is illustrated in Figure 1. F-1

Bacteria. Salmonella typhimurium strains TA1535, hisG46, uvrB, rfa (missense); TA100, hisG46, uvrB, rfa (missense plus R factor); TA1537, hisC3076, uvrB, rfa (frameshift); TA1538, hisD3052, uvrB, rfa (frameshift); and TA98, hisD3052, uvrB, rfa (frameshift plus R factor) were obtained from Dr. Bruce Ames. Standard experimental procedures have been given by Ames et al [2].

Fractions and/or control compounds to be tested were suspended in dimethyl sulfoxide (DMSO) to concentrations in the range of 10-20 mg/ml solids. Normally, the fraction was tested with the plate assay over at least a 1000-fold concentration range. All studies were carried out with parallel

series of plates plus and minus the rat-liver enzyme preparation [4] for metabolic activation. 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 nonmutagenic in the bacterial test system.

Escherichia coli 343/113 (K-12, gal R^{S}18, arg56, nad113) was obtained through the courtesy of Dr. Georges R. Mohn, Frieburg, West Germany. The original culture was maintained frozen (-80°C) in DMSO. Fresh overnight cultures were grown in DIFCO nutrient broth. Media for mutagenesis studies consisted of minimal (1.5% agar) Vogel-Bonner Medium E [5] with 1.0 µg/ml nicotinic acid; other media and experimental details of the liquid suspension tests are described by Mohn [3]. Rat-liver homogenates (S-9) induced with Aroclor 1254 were used in all studies. The bacterial suspension (10^{9} cells/ml) was incubated with the test material, S-9, and the NADPH generating system for 180 min in the dark with shaking. Reversion at arg locus ($arg^{-} \rightarrow arg^{+}$) and forward mutation at gal locus (gal^{+}) were monitored.

RESULTS AND DISCUSSION

To demonstrate the applicability of the coupled analytical-biological approach, we tested primary fractions from a number of crude products from various fossil-fuel technologies with the Salmonella histidine reversion assay.

The unfractionated material was extremely toxic and was not suitable for a mutagenicity assay. However, the fractionated material was assayed with various tester strains and results are presented in Table 1. The first T-1 strain, TA1535, has a base-pair change in the histidine gene and can detect

mutagens that induce base-pair substitutions. The other two, TA1537 and TA1538 are frameshift mutations and will revert to wild-type only with frameshift mutagens. Mutagenic activity was noticed (TA1537 and TA1538) with the basic, ether soluble fraction and the neutral fraction that are expected to contain aromatic amine and poly aromatic hydrocarbons respectively. Metabolic activation with Aroclor induced S-9 was included in the assay. The sensitive tester strains (TA98 and TA100) that contained ampicillin resistance 'R' factor were used in determination of the mutagenic potential of various synfuel fractions. The mutagenic index (specific activity, rev/mg) was determined from the slope value of the linear portion of a dose response curve.

The crude oil exhibited mutagenic activities as assayed with TA98 in a number of fractions; however, because of the actual distribution of the material in the fractionation scheme, the bulk of the impact on the sample as a whole comes from the neutral (PAH) fraction. The results from the syncrude show an increase in total activity over the crude oil. Syncrude exhibited high-spcific-activity components in the basic fractions, B_{Ia} and B_E; and considerable activity, again, in the neutral fraction. Since the original neutral fraction was too toxic to test, it was subfractionated, and the individual values are listed in Table 3. We have assumed that the most accurate measure of the total potential of the neutral fraction is the sum of the tested subfractions. T-3

Results obtained with shale oil were illustrated in Table 4. Data are given for the frameshift strain TA98 with metabolic activation with enzyme preparations from Aroclor 1254-induced rats. The shale-oil samples contained T-4

significant activity in the neutral fractions and the basic, ether-soluble fraction. The neutral portion was subfractionated and the subfractions were assayed for mutagenicity (Table 5). Note that the sum of activities from the neutral subfractions corresponds to the value obtained from the unfractionated neutral material. This supports the additivity of mutagenic potential of the fractions to determine the mutagenicity of the complex original material. T-5

In order to validate the mutagenicity results obtained from the Salmonella histidine-reversion system, we extended the treatment with the selected mutagenic fractions from synfuel A to the E. coli 343/113 system of Mohn [3]. The results are shown in Table 6. T-6

The results obtained in the forward- (gal^+) and reverse-mutation (arg^+) assays with E. coli support the results obtained with Salmonella. Both the basic fraction and the neutral subfraction are mutagenic upon metabolic activation with Aroclor-induced rat-liver homogenate (S-9). The basic fraction was toxic at higher dose points (above 1000 µg) for the induction of gal^+ and arg^+ mutants.

Mutagenicity of organic components. Chemical analyses of synthetic fuel materials [6] and predictions based on work with tobacco smoke condensates [7, 8] give a general view of the organic components of the various fractions. Based on these predictions, we list a selected group (Table 7a and b) of organic compounds pertinent to synthetic fuels and the preliminary results on mutagenicity in the Salmonella histidine-reversion system. The major mutagenic components appear to be heterocyclic nitrogen compounds, aromatic amines, and polycyclic aromatic hydrocarbons. T-7a&b

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. However, we are attempting to verify the significance of the Salmonella and E. coli data by extending a selected suite of synthetic fuel samples to other genetic assays including higher organisms. The use of the mutagenicity data as a prescreen for carcinogenesis may also be of value, in a qualitative sense.

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TABLE 1

MUTAGENIC ACTIVITY OF FRACTIONS FROM COED SYNCRUDE[a]

		Revertants/plate-relative response[b]		
		Strain		
		TA1535	TA1537	TA1538
1.	NaOH, Insol.	-	-	-
2.	Weak Acids, Insol.	-	-	-
3.	Weak Acids, Et_2O Sol.	-	-	-
4.	Strong Acids, Insol.	-	-	-
5.	Strong Acids, Et_2O, Sol.	-	-	-
6.	Strong Acids, H_2O, Sol.	-	*	-
7.	Bases, Insol. (a)	-	-	-
8.	Bases, Insol. (b)	-	-	-
9.	Bases, Et_2O, Sol.	-	**	**
10.	Bases, H_2O, Sol.	-	-	-
	Neutrals			
11.	Hexane	-	**	**
12.	Hexane/Benzene	-	**	**
13.	Benzene/Ether	-	**	**
14.	Methanol	-	-	-

[a]Results obtained with metabolic activation utilizing a rat liver preparation induced with Aroclor 1254.

[b]- denotes no response over background.

*denotes approximately 100 colonies over background.

**denotes approximately 1000 colonies over background (at most effective volume added.

TABLE 2

MUTAGENICITY ASSAY OF FRACTIONATED CRUDE OILS

FRACTION	SYNCRUDE (COED)			CRUDE OIL		
	% OF TOTAL	rev/mg FRACTION	rev/mg TOTAL	% OF TOTAL	rev/mg FRACTION	rev/mg TOTAL
NaOH INSOLUBLE	1.0	0	--	2.9	0	--
WEAK ACIDS, INSOLUBLE	0.1	0	--	0.2	0	--
WEAK ACIDS, ETHER SOLUBLE	1.8	0	--	0.8	0	--
STRONG ACIDS, INSOLUBLE	0.1	0	--	0.2	0	--
STRONG ACIDS, ETHER SOLUBLE	0.9	0	--	0.5	115	<1
STRONG ACIDS, WATER SOLUBLE	0.4	0	--	0.1	236	<1
BASES, INSOLUBLE (A)	0.2	8300	17	0.4	0	--
BASES, INSOLUBLE (B)	0.2	0	--	0.1	0	--
BASES, ETHER SOLUBLE	2.6	1500	39	0.2	175	<1
BASES, WATER SOLUBLE	0.4	0	--	0.1	0	--
NEUTRAL	82.3	**559	460	80.7	**90	73
TOTAL	91.8	--	516	86.2	--	76

**DETERMINED FROM THE TOTAL OF FRACTIONATED NEUTRALS.

TABLE 3

MUTAGENICITY ASSAY OF FRACTIONATED NEUTRALS
(CRUDE OILS)

FRACTION		SYNCRUDE (COED) % OF NEUTRALS	SYNCRUDE (COED) rev/mg FRACTION	SYNCRUDE (COED) rev/mg TOTAL	CRUDE OIL % OF NEUTRALS	CRUDE OIL rev/mg FRACTION	CRUDE OIL rev/mg TOTAL
FRACTIONATED NEUTRALS							
HEXANE	A	87.1	455	396	82.0	92	75
	B	2.6	3100	81	2.0	50	1
	C	1.1	760	8	0.6	168	1
HEXANE/BENZENE	A	1.6	2120	34	3.6	150	5
	B	0.7	2400	17	0.7	254	2
	C	0.6	0	---	0.5	70	<1
BENZENE/ETHER	A	4.1	0	---	3.5	32	1
	B	0.4	200	1	0.3	53	<1
	C	0.2	160	<1	0.2	Not Tested	--
Methanol	A	1.1	1520	18	2.4	32	1
	B	0.4	400	2	0.4	82	<1
	C	0.1	300	<1	0.2	74	<1
$MeCl_2$		0.2	200	<1	----	---	--
SUBTOTAL (NEUTRALS)		100.2	----	559	96.4	----	90
NEUTRAL FRACTION		82.3	TOXIC	----	86.2	TOXIC	--

TABLE 4

DISTRIBUTION OF MUTAGENIC ACTIVITY IN FRACTIONS OF AQUEOUS SAMPLE

	Shale-oil product water		
Fraction	Relative weight % 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
Neutral	2.4	52	1
Total			68

TABLE 5

MUTAGENICITY ASSAY OF FRACTIONATED NEUTRALS FROM SHALE OIL

FRACTION		SHALE OIL % OF TOTAL	FRACTION rev/mg	TOTAL rev/mg
FRACTIONATED NEUTRALS[2]		%-NEUTRALS		
HEXANE	A	58.7	40	23
	B	2.1	625	13
	C	1.3	750	10
HEXANE/BENZENE	A	4.4	238	10
	B	1.9	340	6
	C	1.4	320	4
BENZENE/ETHER	A	12.4	65	8
	B	2.2	142	3
	C	1.3	253	3
METHANOL	A	15.1	179	27
	B	0.5	684	3
	C	0.9	263	2
$MeCl_2$		----	---	---
SUBTOTAL (NEUTRALS)		102.2	---	112
NEUTRAL FRACTION		86.7	109	97

TABLE 6

MUTAGENICITY OF SYNFUEL FRACTIONS IN E. COLI

Treatment	Concentration	Mutants/10^7 survivors	
		arg^+	gal^+
S-9 control*	-	2.04	25.03
9. B_E	100	2.31	15.84
	200	4.44	32.20
14. Methanol	100	3.00	32.78
	1000	49.61	84.10
Dimethylnitrosamine (positive control)	2000	103.00	-

*Average from two independent experiments.

TABLE 7A

MUTAGENICITY OF ORGANIC CONSTITUENTS IDENTIFIED OR SUSPECTED TO OCCUR IN BASIC FRACTION

	Compound		Strain (TA-)	Activation[a] system	his$^+$ rev/100µg	Mutagen
1.	PYRIDINES [13][b]		98 & 100	Ui & Ar	0	-
	METHYL PYRIDINE (PICOLINE)	[3]	98 & 100	Ui & Ar	0	-
	ETHYL PYRIDINE	[2]	98 & 100	Ui & Ar	0	-
	DIMETHYL PYRIDINE (LUTIDINE)	[6]	98 & 100	Ui & Ar	0	-
	TRIMETHYL PYRIDINE	[1]	98 & 100	Ui & Ar	0	-
	BI PYRIDINE	[1]	98 & 100	Ui & Ar	0	-
2.	ANILINES	[7]				
	ETHYL ANILINE	[2]	98 & 100	Ui & Ar	0	-
	DIMETHYL ANILINE (XYLIDINE)	[3]	98 & 100	Ui & Ar	0	-
	2,5-DIMETHYL ANILINE	[1]	98	Ui	34	+
	TRIMETHYL ANILINE	[1]	98 & 100	Ui & Ar	0	-
3.	Quinolines	[8]				
	QUINOLINE	[1]	98	Ar	55	+
	7-METHYL QUINOLINE	[1]	100	Ar	150	+
	8-METHYL QUINOLINE	[1]	100	Ar	80	+
	2,6-DIMETHYL QUINOLINE	[1]	98 & 100	Ui & Ar	0	-
	8-HYDROXY QUINOLINE	[1]	100	Ar	800	+
	8-AMINO QUINOLINE	[1]	1537	ØB	5000	+
	8-NITRO	[1]	100	ØB	300	-
	ISO QUINOLINE	[1]	98 & 100	Ar	0	-
4.	PIPERIDINES	[2]				
	DIMETHYL PIPERIDINES	[2]	98 & 100	Ui & Ar	0	-
5.	MISCELLANEOUS	[6]				
	ACRIDINE	[1]	1537	--	80	+
	α-NAPHTHYLAMINE	[1]	100	Ar	466	+
	DIPHENYLAMINE	[1]	98 & 100	Ui & Ar	0	-
	DIMETHYL QUINOXYLINE	[2]	100	Ui & Ar	0	-
	CARBAZOLE	[1]	100	Ui & Ar	0	-

TABLE 7B

MUTAGENICITY OF ORGANIC CONSTITUENTS IDENTIFIED OR SUSPECTED TO OCCUR IN NEUTRAL FRACTION

	COMPOUND	STRAIN (TA-)	ACTIVATION[a] SYSTEM	his$^+$ REV/100µg	MUTAGEN
1.	ANTHRACENES [8][b]				
	ANTHRACENE	100	Ar	0	-
	BENZ(a)ANTHRACENE	100	Ar	663	+
	BENZ(b)ANTHRACENE	100	Ar	2274	+
	7,12-DIMETHYL BENZ(a)ANTHRACENE	100	Ar	1465	+
	1,2,3,4-DIBENZANTHRACENE	100	Ar	799	+
	1,2,5,6-DIBENZANTHRACENE	100	Ar	5304	+
	9-METHYL ANTHRACENE	98	Ar	86	+
	2-AMINO ANTHRACENE	100	Ar	5300	+
2.	PYRENES [2]				
	PYRENE	1537	Ar	73	+
	BENZO(a)PYRENE	98	Ar	4133	+
3.	FLUORENES [3]				
	FLUORENE	100	Ar	0	-
	2,3-BENZOFLUORENE	100	Ar	858	+
	9-METHYLFLUORENE	100	Ar	0	-
4.	MISCELLANEOUS [6]				
	CHRYSENE	100	Ar	1355	+
	FLUORANTHENE	98	Ar	13	+
	PHENANTHRENE	98	Ar	56	+
	BIPHENYL	100	Ar	0	-
	TRIPHENYLENE	98	Ar	990	+
	NAPHTHYLENE	98, 100	Ar, Ui	0	-

[a]Metabolic activation with Un-induced (Ui) or Aroclor (Ar) or Phenobarbital (ØB) induced rat liver homogenate (S-9) was included.

Number of derivatives tested was given in parenthesis.

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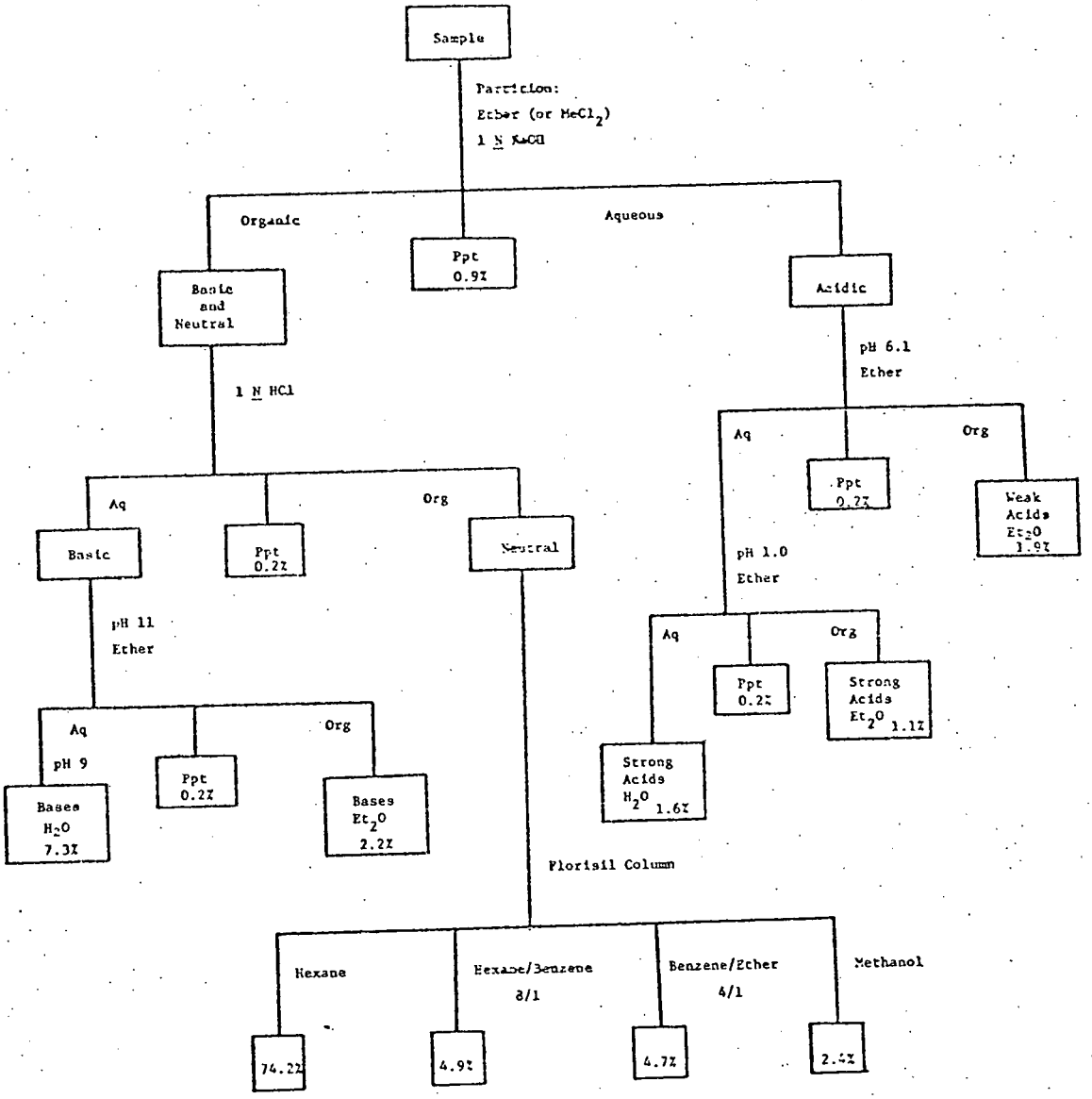

FRACTIONATION SCHEME APPLIED TO PRODUCTS FROM SYNTHETIC FUEL INDUSTRY

FIGURE 1