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MUTAGENICITY OF NITROGEN COMPOUNDS FROM SYNTHETIC CRUDE OILS: COLLECTION, SEPARATION AND BIOLOGICAL TESTING

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DISCLAIMER

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INTRODUCTION

Short-term mutagenesis assays have been utilized to characterize complex environmental mixtures for the possible health effects. These bioassays are intended to (1) serve as predictors of long-range health effects, (2) guide chemical separation procedures for the isolation and concentration of biologically active materials, (3) identify chemical agents responsible for the biological activity and (4) determine priorities for further and extensive testing. Organic extraction coupled with chemical class fractionation is a prerequisite for most of these assays.

Our emphasis has been mainly in evaluating various test materials from the newly emerging synfuel technologies. The bacterial mutagenicity assay developed by Ames [1], utilizes certain well-characterized histidine mutants of Salmonella typhimurium. We have previously reported the application of class chemical separation procedure developed by Swain-Stedman [2] for evaluating certain coal-derived [3] and shale-derived [4] oils. In an effort to minimize chemical reactivity during the fractionation procedure, separation with Sephadex LH-20 gel chromatography [5] was used with several synfuels. Mutagenicity results obtained have indicated the alkaline fractions containing

azaarenes, primary amines and nitro polyaromatics are mutagenic fractions, along with the neutral fraction containing polycyclic aromatic hydrocarbons and nitrogen containing PAH's [10]. Specificity to revert the frameshift strain, TA-1538 and TA-98 [6] and dependence on specific activation systems correlates to the biological activities of these organic constituents.

Sample Description and Source

Samples utilized in this study were supplied by USEPA/USDOE synfuel research materials facility [7]. Process operating conditions at the time of sampling, sampling conditions and sample histories are not sufficiently defined to provide process-specific conclusions. Sample listing (with repository numbers) and source are listed in Table 1. A detailed process description was previously presented [8].

TABLE I.

Description of Synfuel Samples

Repository Number	Sample	Source
A. Petroleum samples		
5301	Wilmington Crude Oil	Bartlesville Energy Technology Center
5305	Recluse Crude Oil	Bartlesville Energy Technology Center
B. Shale derived oils		
4101	Shale Oil ('in situ')	Laramie Energy Technology Center
4601	Paraho Shale Oil	US Navy/Standard Oil Company of Ohio
4602	HDT-Paraho Shale Oil	US Navy/Standard Oil Company of Ohio
C. Coal derived oils		
1701	SRC II-fuel oil	Pittsburgh and Midway Mining Company
1601	H-Coal-dist.-raw	Mobil Research/EPRI
1602	H-Coal dist. HDT-low severity	Mobil Research/EPRI
1603	H-Coal dist. HDT-med. severity	Mobil Research/EPRI
1604	H-Coal dist. HDT-high severity	Mobil Research/EPRI
1801	$ZnCl_2$ dist.	Conoco Coal Development Company

Purpose

The objective of this study has been to identify mutagenic activity in the fractionated synfuel samples, followed by isolation and identification of chemical agents responsible for this activity.

MATERIALS AND METHODS

Chemical Fractionation

The chemical fractionation procedure (Figure 1) was previously described [8]. The procedure involved separation of acidic and basic fractions using liquid/liquid partitioning into ether soluble and insoluble acids and bases (water soluble fractions were generally found inactive in the mutagenicity assays). The neutral fraction was separated using Sephadex LH-20 into aliphatics, aromatic, polyaromatic and polar fractions by isopropanol elution. Solvents were removed by rotary evaporation, the residue was dissolved in dimethyl sulfoxide for mutagenicity assays. The basic fraction was subfractionated using basic alumina and Sephadex LH-20 (Figure 1). The ether soluble base (ESB) fraction was loaded onto the column and eluted with 500 ml benzene (benzene subfraction) followed by 700 ml ethanol. Ethanol was removed by rotary evaporation and the residue was separated further on a Sephadex LH-20 gel column. The column was eluted sequentially with 250 ml of isopropanol (isopropanol subfraction) and 600 ml of acetone (acetone subfraction). The eluting solvent was removed by rotary evaporation.

Mutagenicity Assay

Histidine auxotrophic strain of Salmonella typhimurium TA-98, obtained through the courtesy of Dr. B. N. Ames, Berkeley, CA, was used for these studies. The standard procedure (1) involved addition of bacterial cells (2×10^8) to soft agar containing the fraction being tested and overlay on minimal agar plates. Liver homogenates (S-9 mix) from Aroclor 1254 induced rats was incorporated in each plate to provide for metabolic activation. Activity in revertants per unit weight (rev/mg) of test substance is derived from the slope of the induction curve. Total mutagenic activity of a starting material is computed from the activities of its subfractions following correction for the weight percentages contributed by the subfractions to the starting material.

RESULTS AND DISCUSSION

The crude oils could not be tested for mutagenicity due to their toxic property; when tested the crudes yielded questionable results. In order to overcome this problem and also in obtaining a more homogeneous distribution of sample in the test medium, chemical fractionation was applied. The general procedure was described and illustrated in Figure 1. Recovery and reproducibility have been tested by using triplicate samples of shale oil and crude oil. Chemical recovery and reproducibility is adequate for biological testing purposes, even though recoveries are unacceptably low. The losses are caused by volatile matter which would be hard to apply in the biotesting.

Table 2 illustrates distribution of mutagenic activity in petroleum oils, shale oil and coal-derived oils. The acidic fractions were inactive in the mutagenicity assays. All the activity was recovered from the basic and neutral fractions which varied from sample to sample. The petroleum samples contained all the activity in the neutral fraction which constituted weak mutagenic activities. The shale oil samples that are rich in nitrogen content exhibited activity distributed between the neutral and the basic fractions. Significant mutagenic activity was also observed with the coal-derived oils. Both the shale oil and coal-derived oils were relatively more mutagenic than the petroleum samples.

TABLE 2

DISTRIBUTION OF MUTAGENIC ACTIVITY IN SYNFUELS[a]

			Mutagenic Activity			
			Percent Distribution			
		Total (Rev/mg)[b]	Neutral	Acids	Bases	Other
A. Petroleum Samples						
5301	Wilmington Crude Oil	5	100	0	0	0
5305	Recluse Crude Oil	6	100	0	0	0
B. Shale-derived oils						
4101	Shale Oil ('in situ')	178	54	2	42	2
4601	Paraho Shale Oil	390	31	0	69	0
4602	HDT-Paraho Shale Oil	0	0	0	0	0
C. Coal-derived oils						
1701	SRC-II-fuel oil	1000	65	0	35	0
1601	H-Coal dist.-raw	350	63	0	37	0
1602	H-Coal dist. HDT-low	540	100	0	0	0
1603	H-Coal dist. HDT-med.	210	-	-	-	-
1604	H-Coal dist. HDT-high	0	0	0	0	0
1801	$ZnCl_2$ dist.	530	100	0	0	0

[a] references 8, 9.

[b] determined from the linear portion of a dose-response curve with strain TA-98.

Effects of hydrotreatment seem to eliminate the mutagenic activity as evidenced from the results obtained with HDT-H-Coal distillates. The high severity hydrotreatment has completely eliminated mutagenic activity of H-Coal sample while the medium and low severity treatments were relatively less effective. $ZnCl_2$ catalyzed distillation seems to have eliminated the alkaline mutagens in the coal-derived oil.

Presence of mutagenic activity in the basic and neutral fractions has led us to examine these fractions to isolate and identify chemical agents responsible for the biological activity. The basic fraction was subfractionated using basic alumina column and Sephadex LH-20 method. Biological activity was recovered in the ultimate acetone fraction that comprised of approximately 10% of the starting ESB-fraction. Results obtained with subfractionated SRC-II-ESB and Shale oil-ESB are given in Table 3. The acetone fractions with high specific activities can be applied for biotesting in other genetic systems as well as for chemical analysis [11]. Azaarenes and primary aromatic amines are the suspected organic constituents of this fraction causing the mutagenic activity.

TABLE 3

ISOLATION OF MUTAGENIC ACTIVITY FROM THE BASIC FRACTION (ESB)

	SRC-II-ESB (1701)			SHALE OIL-ESB (4101)		
	Relative Weight (%)	Specific Activity (Rev/mg)	Weighted Activity (Rev/mg)	Relative Weight (%)	Specific Activity (Rev/mg)	Weighted Activity (Rev/mg)
ESB	—	0	14,000	—	—	2,500
Benzene	74	0	0	77	0	0
Isopropanol	6	400	24	12	0	0
Acetone	15	68,000	10,200	9	20,000	
TOTAL	95		10,224	98		1,800

TABLE 4

MUTAGENIC ACTIVITIES OF NEUTRAL SUBFRACTIONS OF A COAL-DERIVED OIL AND A SHALE OIL[a]

Subfraction	Specific Activity (rev/mg) Coal-Derived Oil	Shale Oil
Aliphatic (AL)	0	0
PAH(I)	1390	120
Neutral N-PAH (II)	3250	0
Polar (III)	3380	1100

[a]taken from reference 10.

Chemical separation and analysis of the neutral fraction suggested polyaromatic hydrocarbons (PAH's) alkylated PAH's [12] and nitrogen-PAH's [10] as the possible mutagenic agents. Mutagenic activity of PAH's and alkylated PAH's appeared to increase with the ring size (Figure 2). The aliphatic constituents of coal-derived materials were not mutagenic while the nitrogen PAH fraction obtained from coal oil [10] had a specific activity more than twice that of the PAH fraction (Table 4). The nitrogen-PAH fraction from a shale-derived oil was not mutagenic.

CONCLUSIONS

1. Short-term bioassay such as the Ames Salmonella histidine reversion assay can be effectively applied to the complex environmental samples.
2. Proper chemical extraction and fractionation methods should be coupled to the assay.
3. The Shale- and Coal-derived oils are relatively more mutagenic than petroleum crude.
4. Mutagenic activity was mainly associated with the basic and neutral fraction.
5. Azaarenes, aromatic amines, PAH's, alkylated PAH's and nitrogen-PAH's are the suspect organic constituents of these fractions, causing the mutagenic activity.

SUMMARY

In order to determine the long range health effects such as carcinogenicity/mutagenicity/teratogenicity/toxicity, associated with the newly emerging energy technologies, we have utilized the Ames Salmonella assay to evaluate mutagenic properties of synthetic fuels. Coupling with class fractionation was necessary. Organic extraction and liquid/liquid partitioning was used to separate acidic and basic fraction. The neutral material was separated using Sephadex LH-20 gel filtration into saturated and aromatic fractions of various ring sizes. The alkaline fraction was subfractionated eluting with benzene and ethanol on a basic alumina column and then with isopropanol and acetone using a Sephadex LH-20 gel column. The frameshift strain TA-98 was utilized along with Aroclor-induced rat liver homogenate (S-9 mix) for the mutagenicity assay. The natural crude oils were slightly mutagenic, the polynucleararomatics constituting the activity, while the coal-derived fuels indicated mutagenicity associated with alkaline constituents as well as polyaromatics. Hydrotreated coal (H-coal, HDT) or Shale (Paraho-Shale oil, HDT) derived fuels were not mutagenic. Ninety percent of the mutagenic activity in alkaline fraction was recovered in the acetone subfraction. High resolution spectroscopy of this fraction indicates polycyclic aromatic primary amines along with azaarenes as organic constituents responsible for the mutagenic activity associated with shale- and coal-derived fuels.

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FIGURE LEGEND

Figure 2. Mutagenicity of PAH and Alk-PAH subfraction.

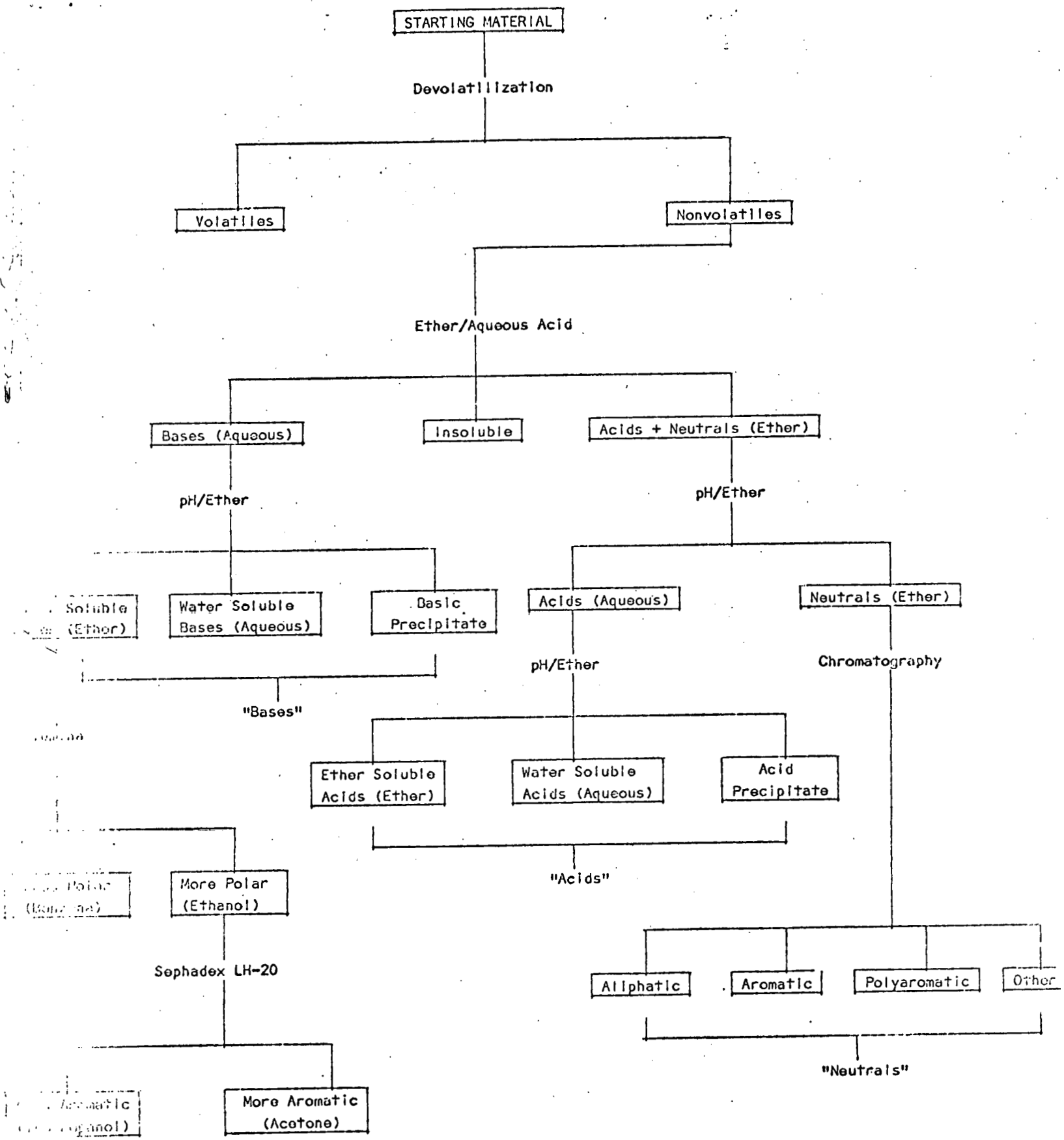

Figure 1. Chemical Class Fractionation Procedure for Chemistry and Biotesting.
Material , Step, (Phase)

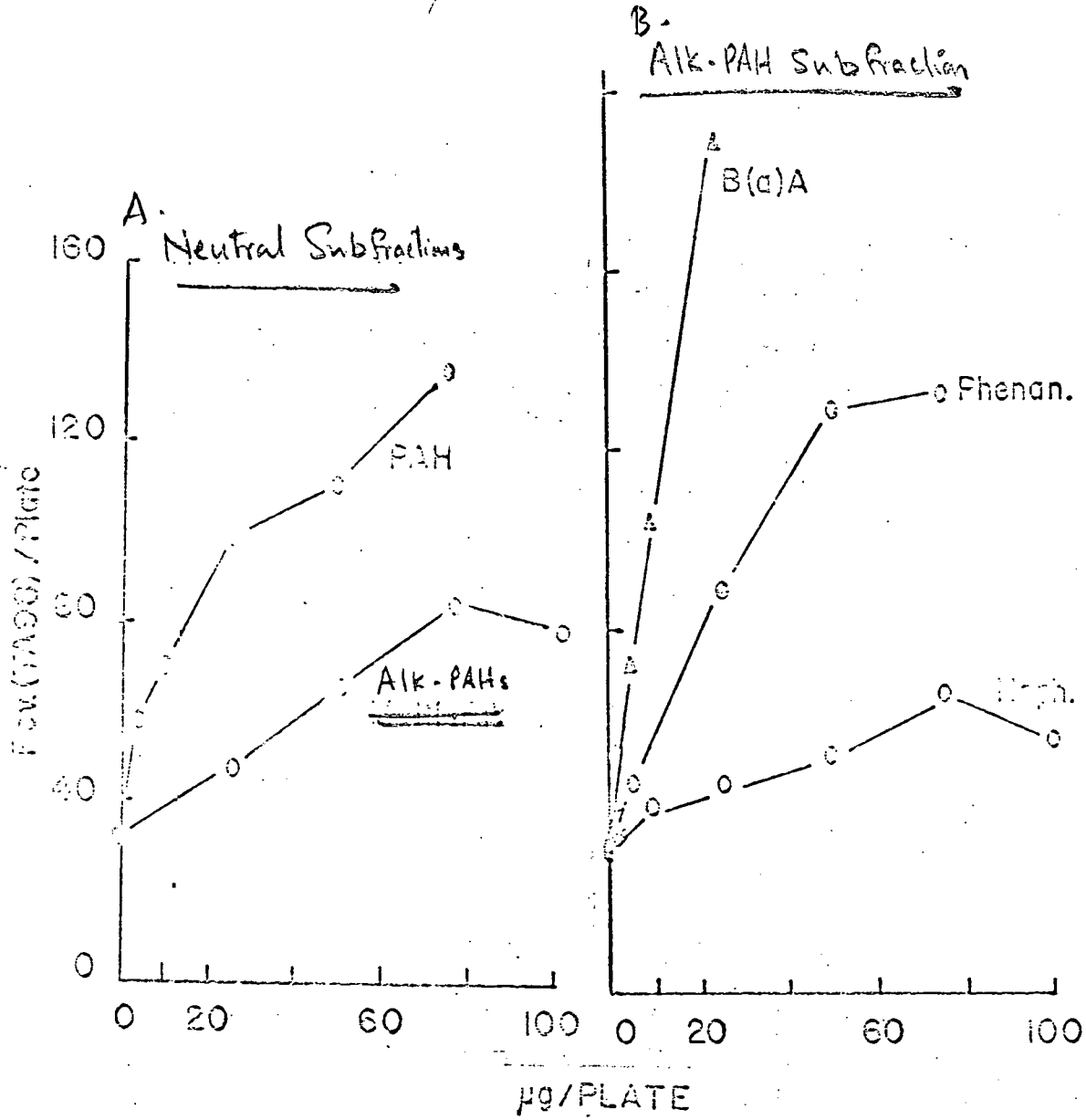
A.
Neutral Subfractions
160
120
80
40
0
Rev. (TA98) / Plate
PAH
Alk-PAHs
0
20
60
100
B.
Alk-PAH Subfraction
B(a)A
Phenan.
Fluor.
0
20
60
100
µg/PLATE

Fig. 2