PNL-SA-8439

CONF-800334--21

TOXICOLOGIC STUDIES OF SRC MATERIALS

D.D. Mahlum, R.A. Pelroy, H. Drucker
Biology Department
B.W. Wilson
Physical Science Department
Pacific Northwest Laboratory
Richland, Washington 99352
Operated by Battelle Memorial Institute

also

M.J. Massey
Environmental Research & Technology Inc.
Pittsburgh, Pennsylvania

and

D.K. Schmalzer
Gulf Mineral Resources
Denver, Colorado

February 1980

Prepared for the 2nd DOE Environmental Control Symposium
Sheraton International Conference Center
Reston, VA, March 19, 1980
To be published in proceedings of symposium

Prepared for the U.S. Department of Energy
under Contract EY-76-C-06-1830

DISCLAIMER

TOXICOLOGIC STUDIES OF SRC MATERIALS

by

D.D. Mahlum, R.A. Pelroy, H. Drucker
Biology Department

and

B.W. Wilson
Physical Science Department
Pacific Northwest Laboratory
Richland, Washington 99352
Operated by Battelle Memorial Institute

also

M.J. Massey
Environmental Research & Technology, Inc.
Pittsburgh, Pennsylvania

and

D.K. Schmalzer
Gulf Mineral Resources
Denver, Colorado

Prepared for the U.S. Department of Energy
under Contract EY-76-C-06-1830

TOXICOLOGIC STUDIES OF SRC MATERIALS

D.D. Mahlum, R.A. Pelroy, B.W. Wilson, and H. Drucker
Pacific Northwest Laboratory

M.J. Massey - ERT
D.K. Schmalzer - Gulf
Environmental Research & Technology, Inc.
Gulf Mineral Resources Co.

In light of the energy crisis which the U.S. faces, it is becoming imperative to develop new energy technologies in as rapid a manner as possible. In order for these new technologies to develop in the most propitious manner, it is necessary to perform studies to determine potential health and environmental effects associated with the technology. However, it is sometimes difficult to perform these studies with materials which are meaningful to the final process that is developed. Thus, a "Catch 22" situation develops. You have to have the technology before appropriate materials can be obtained; yet you need the health and environmental studies before the technology is deemed acceptable. We are going to describe an approach which has been taken in the solvent refined coal area to provide meaningful health effects data to the technology in a time-frame which permits technology changes to ameliorate potential problem areas.

We at the Pacific Northwest Laboratory have been engaged in a broadly based toxicologic program dealing with SRC materials. These studies have included 1) microbial mutagenesis, 2) in vitro mammalian cell toxicity and transformation assays, 3) epidermal carcinogenesis (skin painting), 4) acute and subchronic oral toxicity, 5) developmental toxicity, 6) dominant lethal assays, 7) inhalation toxicity, and 8) dosimetry and metabolism. We have approached the study in two concomitant modes. In the first mode we have used chemical characterization and plant operational data to select materials to be evaluated using a tier approach. In this mode, materials are first assayed using microbial systems. On the basis of the results of the microbial assays, materials are selected for study in mammalian cell culture. The results generated in the cellular systems are used as selection criteria for materials to be used in animal assays, particularly the assays for carcinogenesis. In the second mode, materials considered to be of prime importance to occupational or environmental health are entered directly into animal systems for study of acute, subchronic, mutagenic, and developmental effects. Long-term effect studies are then designed using the results from these whole-animal and the cellular assays, and the chemical characterization and plant operational data. At each level of testing, we have included other materials for comparative purposes. These materials which include shale oil, petroleum crudes, other fossil derived materials, and pure known chemical mutagens and carcinogens help us in placing results with SRC materials in a more meaningful perspective.

We have in our program attempted to establish a balance between standardized biological testing and research. The use of standard testing protocols permits us to obtain information necessary for the evaluation of the potential health hazards of a number of important materials in order to meet regulatory demands. Research, on the other hand, enables us to obtain a better understanding of the nature of potentially harmful materials as well as to obtain data which will permit a better extrapolation of the experimental data demand. The research effort also allows us to explore potential ways of ameliorating the adverse effects of such materials.

Prepared for the U.S. Department of Energy
under Contract EY-76-C-06-1830

The types of materials encountered in coal liquefaction processes are generally complex mixtures which may behave quite differently in biological systems than do individual constituents. Interactions among constituents may result in both antagonistic and synergistic effects, the sum of which may or may not be predicted from the individual materials. Our philosophy, therefore, is to chemically characterize SRC materials (raw products, process stream materials, solid wastes, etc) which are important in considerations of health and environmental consequences of production, storage, transport, accidental spills, and maintenance. We then characterize these mixtures biologically using cellular and animal systems and relate biologic activity to chemical composition. Further identification of the components producing biological activity is performed through iterative cycles consisting of chemical and physical fractionation accompanied by bioassay of the fractions obtained.

I would like to describe in some detail one facet of our program to: 1) illustrate a part of our approach to the toxicologic study of complex coal liquids, 2) show the benefit of interactions between biologists, chemists, and process engineers, and 3) demonstrate the necessity of evaluation and reevaluation of the relevance and representativeness to demonstration/commercial operation of the samples being evaluated. I will use our microbial mutagenesis studies since they illustrate these points in a concise way.

The materials used in these studies have been obtained from the SRC pilot plant at Ft. Lewis, Washington which is operated by the Pittsburgh & Midway Coal Mining Co., Inc. for the Department of Energy. Light oil (LO), wash solvent (WS), and process solvent (PS) were obtained from the SRC-I process and light, middle, and heavy distillates (LD, MD, and HD) from the SRC-II process. The boiling point ranges and specific gravity ranges for these materials are given in Table 1. The materials in all cases were obtained during equilibrium run conditions when the processes were being operated for determination of material balances. It should be noted that these materials are of pilot plant origin and may or may not represent materials to be found in demonstration or commercial applications. We will discuss this aspect later in the paper.

Table 1

Boiling Point Ranges of SRC Materials
Used in Biological Experiments

Process	Material	Boiling Range (°F)	Density
SRC-I	Light Oil	ambient to 380	0.72
	Wash Solvent	380 to 480	0.96
	Process Solvent	480 to 850	1.04
SRC-II	Light Distillate	134 to 353	0.82
	Middle Distillate	366 to 541	0.99
	Heavy Distillate	570 to 850	1.10

To illustrate how biologic evaluation can be integrated with chemical characterization and with process operations, I would like to present some data which have been obtained using the Ames Salmonella System. 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.

The mutagenic activity of a number of crude materials are shown in abbreviated form in Table 2. It can be seen that of the major process stream materials from the SRC-I and SRC-II processes, PS and HD (high boiling point materials) contain substantial mutagenic activity. The LO, WS, LD, and MD were without activity. The known chemical carcinogen, benzo(a)pyrene (BaP), shows approximately 3X the activity of heavy distillate (HD) while another well-known chemical carcinogen, 2-aminoanthracene (2-AA), is about 100 times as active. Raw shale oil shows a small degree of activity while crude petroleum showed no activity in the Ames system.

Table 2

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

Materials	Revertants/μg of Material
SRC-I	
Process solvent	12.3 + 1.9
Wash solvent	<0.01
Light oil	<0.01
SRC-II	
Heavy Distillate	40.0 + 23
Middle distillate	<0.01
Light distillate	<0.01
Shale Oil	
Paraho-16	0.60 ± 0.19
Paraho-504	0.59 ± 0.13
Livermore LO1	0.65 ± 0.22
Crude Petroleum	
Prudhoe Bay	<0.01
Wilmington	<0.01
Pure Carcinogens	
Benzo(a)pyrene	114 ± 5
2-Aminoanthracene	5430 ± 394

In order to determine the classes of materials responsible for the mutagenic activity, fractions were prepared from PS and HD using the solvent extraction procedures similar to that employed by Swain and Stedman for fractionation of tobacco-smoke condensates, and widely used for chemical class fractionation of environmentally complex mixtures. Since PS and HD only varied in their quantitative response in the Ames system, the remainder of this report will generally use HD to illustrate the approach we have used in identifying important materials. In addition to yielding the expected acidic, basic, and neutral fractions, fractionation of HD also yielded basic and neutral tar fractions. The relative amounts of material in each fraction are shown in

Figure 1. When these fractions were assayed using the Ames system, the highest specific activity (number of revertants per microgram of material) was found in the basic fraction (Table 3). Significant activity was also found in the basic and neutral tar fractions, although the specific activity was about 1/8 to 1/2 of that of the basic fraction. The total mutagenic activity in the basic and neutral tar fractions, however, was greater than that in the basic fraction due to the substantially greater mass of the tars. The acidic and neutral fractions showed essentially no mutagenic activity. Under the conditions of these experiments, the polynuclear aromatic (PNA) fraction did not demonstrate significant mutagenic activity. It is important to note at this point that this occurs not because of the absence of potentially mutagenic components in the PNA fraction but is probably due to the large number of compounds in this material which potentially prevent metabolic activation of mutagenic components.

Table 3

Mutagenic Activity in Fractions of SRC-II Heavy Distillate (HD)

Material	Revertants/μg	% of Total Activity
Heavy distillate, unfractionated	40	100
Basic fraction	400	4
Basic tar	66	16
Neutral tar	108	28
Benzo(a)pyrene	114	---
2-Aminoanthracene	4326	---

The above results led to further studies of the nature of mutagenic materials active in HD. It was felt on the basis of previous chemical analyses that polar nitrogen-containing compounds might be responsible. To test this idea the SRC-II distillates and their basic and tar fractions were analyzed by thin layer chromatography (TLC) using a solvent system designed to separate the polar nitrogen-containing compounds from less-polar constituents. Samples of pure chemicals were also chromatographed to aid in the location and identification of major compound classes known or suspected to be present in HD. Fluorescent areas corresponding to these chemicals were located by illumination with an ultraviolet light. The chromatograms were cut into strips, the strips extracted with a hexane-acetone mixture, and the extracts subjected to study for mutagenic activity.

The separation of the mutagenic constituents from the basic fraction of HD is depicted schematically in Figure 2. The major portion of the activity was found to migrate with Rf values (distance migrated by the test material/distance traveled by the solvent front on a TLC plate) between 0.07 and 0.20. These values are very close to those obtained for aromatic amines, a class of compounds suspected but not previously shown to be present in SRC-II materials. Similar results were obtained when the basic tar or neutral tar fraction of HD (or PS and PS basic and tar fractions) were chromatographed.

Extracts from the mutagenically active regions after thin layer chromatography of heavy distillate and its fractions were subjected to gas chromatographic/mass spectral (GCMS) analysis to identify specific components. Several primary aromatic amines, including aminonaphthalenes (AN), aminoanthracenes (AA, aminophenanthrene (APH), aminopyrenes (AP), and aminochrysenes (AC),

were found to be associated with TLC regions which were mutagenically active. An estimate of the aromatic amine content of the active TLC regions from the HD basic fraction is shown in Figure 3; the relative concentrations of AA, AP, APH, and AC were highest in the regions with the strongest mutagenic activity. With the exception of AN, primary aromatic amines were not found in regions that lacked mutagenic activity. Aminofluorenes (AF) and aminocarbazoles were tentatively identified in active regions from the basic fraction but higher-resolution analysis must be performed to obtain more definite identification. Qualitatively similar results were obtained when the basic or neutral tar fractions were analyzed. Analyses of these materials suggest that the 3- and 4-ring primary amines are important mutagens but that the 2-ring aminonaphthalenes contribute little to mutagenic activity.

Since GCMS analysis showed the presence of aromatic amines in the mutagenically active regions of thin layer chromatograms of the basic, basic tar, and neutral tar fractions of HD, a series of experiments were performed to determine if these amines were responsible for the mutagenic activity. One approach utilized the unique catalytic properties of a purified liver enzyme system called mixed-function amine oxidase (MFAO). This enzyme is specific for the metabolic transformation of primary aromatic amines to a mutagenically active state. The MFAO is inactive with BaP and other polycyclic aromatic hydrocarbons (PAH). Nor does it active the 2-aminonaphthalenes, probably due to the instability of the enzyme product. Previous experiments utilized a mixture of hepatic enzymes (called S9) which activates a wide variety of PAHs; therefore, no distinction could be made between amines and other polycyclic constituents. In this experiment, the basic fraction of HD was again subjected to thin layer chromatography and the various regions tested for mutagenic activity after activation with either MFAO or S9. Again, TLC regions with RF's of approximately 0.08 to 0.20 were found to contain the major portion of the activity when the assay was performed using S9, as had been seen previously. When activation was performed using MFAO, the same distribution of mutagenic activity among the TLC regions was found as with S9. These results thus provide further evidence that aromatic amines are both present and capable of expressing their mutagenic activity in the basic fraction of HD.

The above data were considered as presumptive for the involvement of the primary aromatic amines as causative agents in the mutagenic activity of the basic fraction and of the heavy distillate. A more direct approach was taken by treating HD and its basic fraction with nitrous acid. This procedure diazotizes aromatic amines and renders them nonmutagenic in the Ames system. Thus, disappearance of mutagenic activity in the basic fraction or in the heavy distillate after nitrous acid treatment would provide direct evidence for the importance of this class of compounds for mutagenesis. When this was done with a number of materials, as shown in Figure 4, it can be seen that the mutagenic activity of a pure aromatic amine to aminoanthracene was almost completely lost while the activity of benz(a)pyrene or benzacridine was not affected by the nitrous acid treatment. Moreover, treatment with nitrous acid aliminated most of the activity seen with HD, PS, and their basic fractions. It thus appears that much of the mutagenic activity is probably due to the presence of primary aromatic amines in both the crude material and in the basic fractions.

The data obtained thus far in the Ames System, have been used to guide a number of other biological experiments. For example, a series of mammalian cell culture experiments have been performed using materials which are active

and inactive in the Ames system. The data obtained in these experiments confirm and extend the Ames results. We also have initiated skin painting experiments with SRC materials. Since not all materials could be tested, we have utilized those which have shown substantial activity in the Ames system and have also shown transformation activity in mammalian cell culture experiments. For example, HD was initially selected as an SRC-II material to be tested in skin painting. Since light distillate has been shown to be inactive, we also used it for comparison. These comparative experiments allow us to evaluate the ability of the cellular systems to predict the carcinogenic potential of these crude materials.

Although there is not the space to present the details of the mammalian cell culture and skin painting experiments, a summary of results from the Ames assay, mammalian cell culture studies, and skin painting experiments are presented in Table 4. Examination of the results show that there is relatively good qualitative agreement between the cellular assays and the tumorigenesis data. However, there are quantitative differences. For example, mutagenic activity of 2-aminoanthracene is very high but tumorigenic activity is only moderate. On the other hand, the mutagenic activity of HD is moderate but the tumorigenic activity is high.

As further separations are performed on materials derived from HD, the Ames system is used to determine their biologic activity. Those showing mutagenic activity are then subjected to further chemical characterization using, particularly, gas chromatographic mass spectrometry. Thus, this interaction between use of a simple biological system and chemical characterization will continue to be used to determine if further localization of the activity to certain specific materials can be made.

The data obtained above have also suggested that the amount of biological activity as represented by mutagenesis in the Ames system might be altered by changing process conditions or by adding other steps to the processing. For example, a sample of coal liquids was hydrotreated under either moderate or severe processing conditions. When the treated material was compared to the untreated material using the Ames system, it was found that the mutagenic activity was significantly reduced by both severe and moderate hydrotreating (Fig. 5). These biological results correlate well with changes in chemical composition produced by hydrotreating (for further discussion of the effects of hydrotreating on chemical composition, see the paper by Wilson, Pelroy, and Craun elsewhere in this volume). These data suggest that certain process treatments may be important in altering the biological activity. Thus, biological data obtained early in process development are being used in considerations of ways of altering the development of the technology to make the products more acceptable from a biological and environmental viewpoint.

Since the materials used in the described studies were obtained about two years ago when less information was available concerning the design of the demonstration plant, a detailed review of the pilot plant operation when these samples were obtained, has been performed. Conditions which pertained when the middle and heavy distillate samples were obtained are illustrated in Figure 6. From this sequence of operating conditions, the following factors must be considered relative to the materials used:

1) Samples were taken before fully stable operation was achieved.
2) Feed to the distillation tower at the time of MD and HD sampling included:
   a. Fresh production from 2 days of balance period operation.
   b. Seal flush HD from balance period.
   c. Seal flush HD during 30 hours of plant upset.

d. Seal flush HD and freshly produced material during approximately 30 hours of start-up following plant upset.

3. The coal type (Blackville) is relatively unreactive and of low priority for commercial-scale SRC feed.

Table 4

Comparison of Mutagenic and Carcinogenic Activity for Several Crude Fossil-Derived Materials

Material	Ames Assay	Mammalian Cell Culture	Skin Tumorigenesis
Light distillate	---	---	---
Heavy distillate	++	++	++++
Shale oil	+	+	++
Crude petroleum	--	slight	+
Benzo(a)pyrene	++	++	+++
2-Aminoanthracene	++++	+++	++

This information illustrates that a number of complicating factors may be encountered during sampling of materials and that these factors must be taken into account when extrapolating the data to scaled-up operations. In the case of our studies, this evaluation shows that the materials used actually represented a cross-section of a number of conditions. Thus, the data obtained are judged to have generic significance to scaled-up operations. That is, they are probably good in a qualitative sense, but additional data are needed to ensure that the results can be applied quantitatively to demonstration/commercial operation. Thus, there must be continual interaction among the process engineers, the chemist, and the biologist to ensure that meaningful data are obtained in the right time frame and that these data can be updated as more detailed knowledge of demonstration and commercial designs are obtained. We suggest the need for comparable evaluations for other technologies.

With these cautions in mind, improved sampling procedures have been devised for obtaining materials under specified operating conditions. The sampling procedures take into account elements of the process which may influence rather dramatically the composition of the materials to be obtained. Thus, a higher degree of confidence will be possible when the next set of samples will be even more meaningful to the operation of the demonstration and commercial plants.

Figure Legends

Figure 1. Percentage distribution of fractions obtained by solvent fractionation of heavy distillate.

Figure 2. Thin layer chromatographic separation of mutagenic agents found in the basic fraction of heavy distillate.

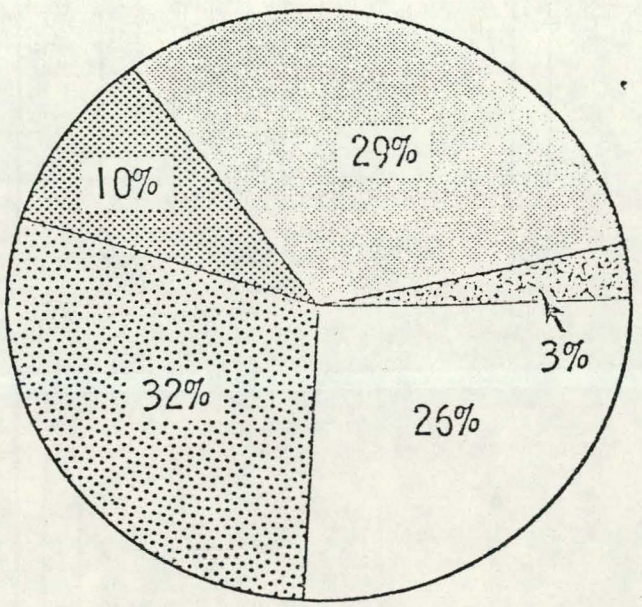
Figure 3. Relative concentrations of aromatic amines and mutagenic activity on thin layer chromatograms of the basic fraction of heavy distillate.

Figure 4. Effect of nitrosation on the mutagenic activity of SRC materials.

Figure 5. Effect of moderate and severe hydrotreating on the mutagenic activity of a coal-derived fuel oil.

Figure 6. Specific history of SRC-II Pilot-plant materials used by PNL in its biomedical studies.

Figure 1



NEUTRAL
BASIC
ACIDIC
INSOLUBLE
PNA

Figure 2

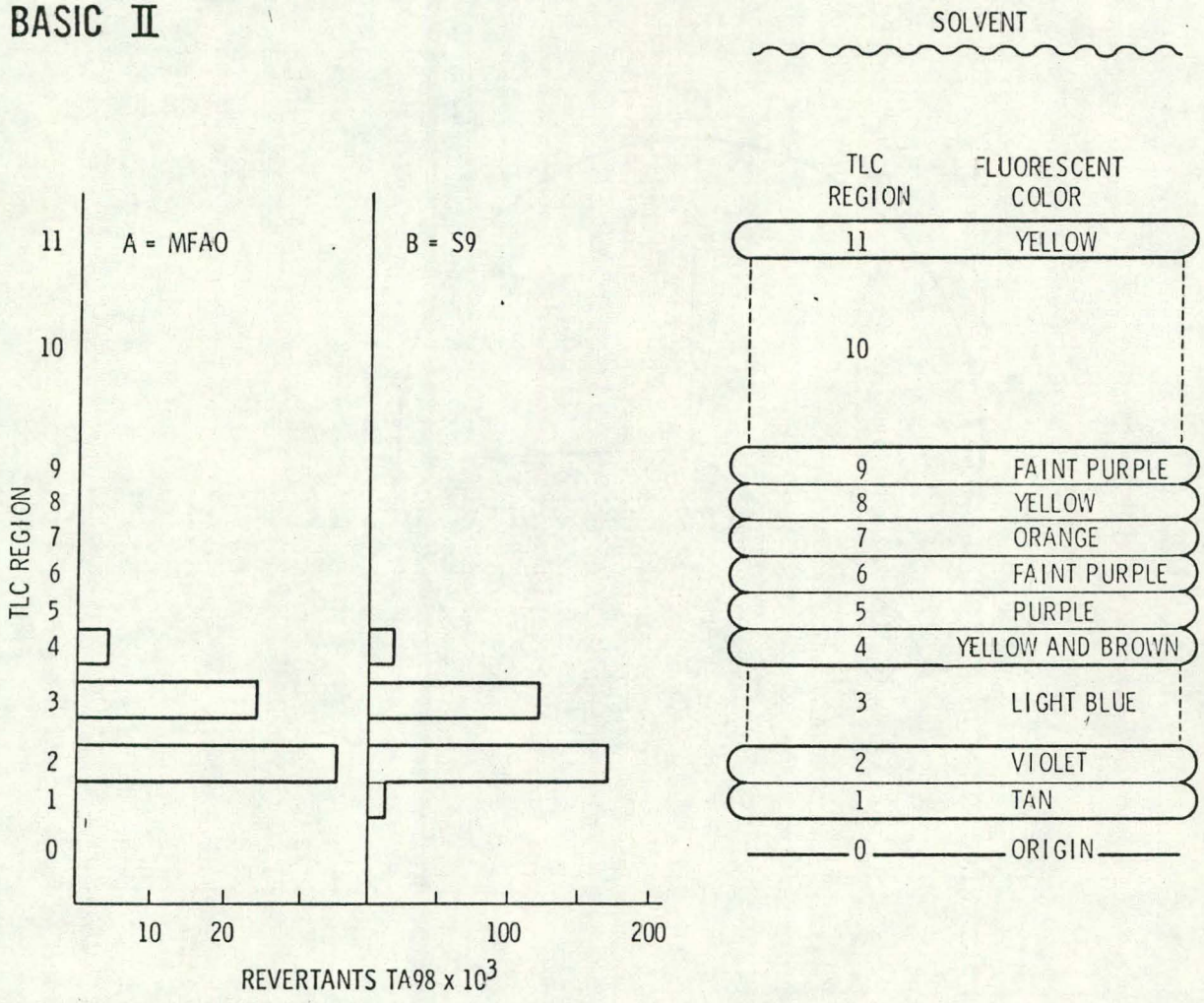

BASIC II
SOLVENT
TLC REGION
FLUORESCENT COLOR
11
YELLOW
10
9
FAINT PURPLE
8
YELLOW
7
ORANGE
6
FAINT PURPLE
5
PURPLE
4
YELLOW AND BROWN
3
LIGHT BLUE
2
VIOLET
1
TAN
0
ORIGIN
A = MFAO
B = S9
TLC REGION
11
10
9
8
7
6
5
4
3
2
1
0
10
20
100
200
REVERTANTS TA98 x 10^3

Figure 3

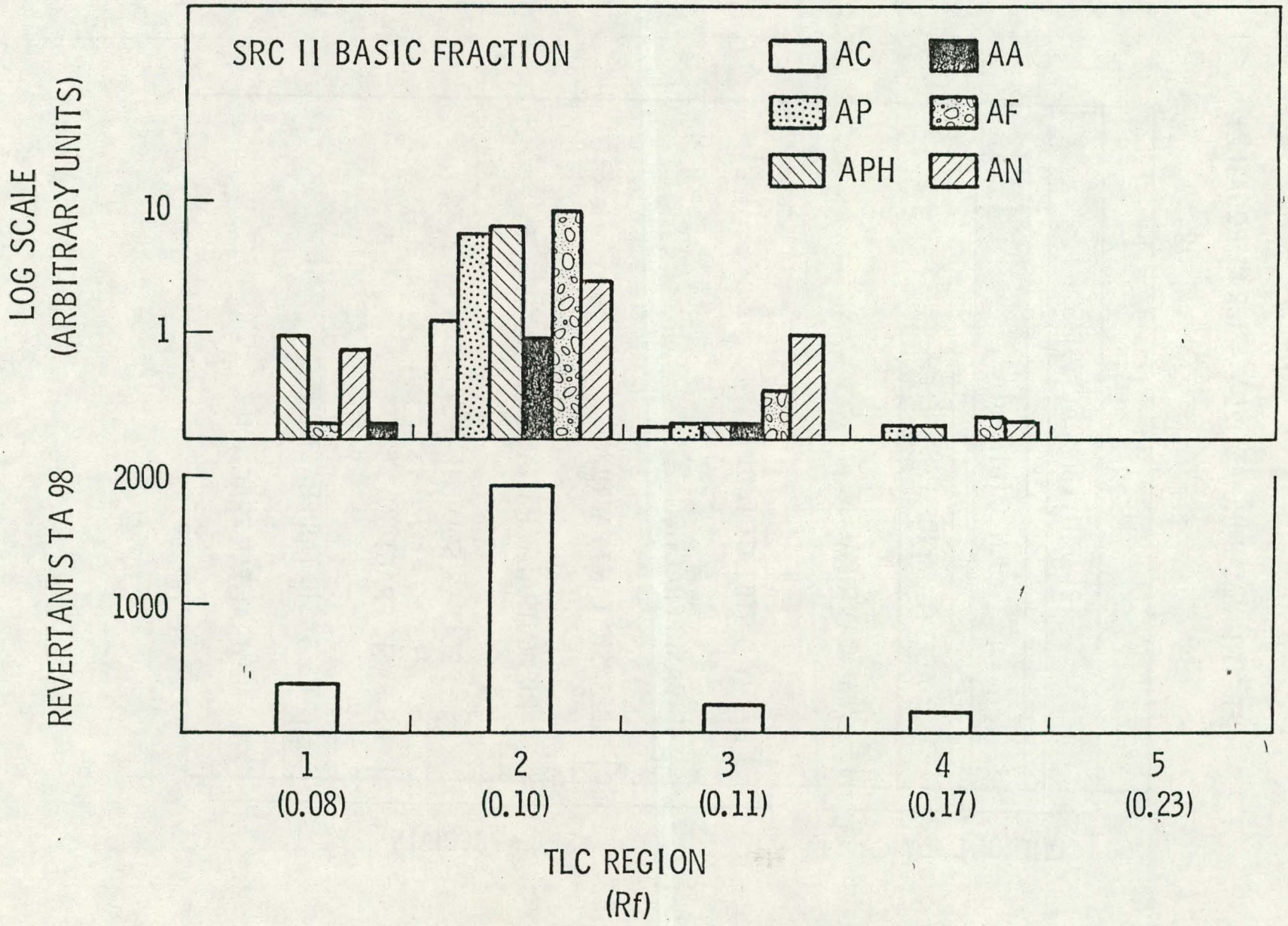

SRC II BASIC FRACTION
AC
AA
AP
AF
APH
AN
LOG SCALE
(ARBITRARY UNITS)
10
1
REVERTANTS TA 98
2000
1000
1
(0.08)
2
(0.10)
3
(0.11)
4
(0.17)
5
(0.23)
TLC REGION
(Rf)

Figure 4

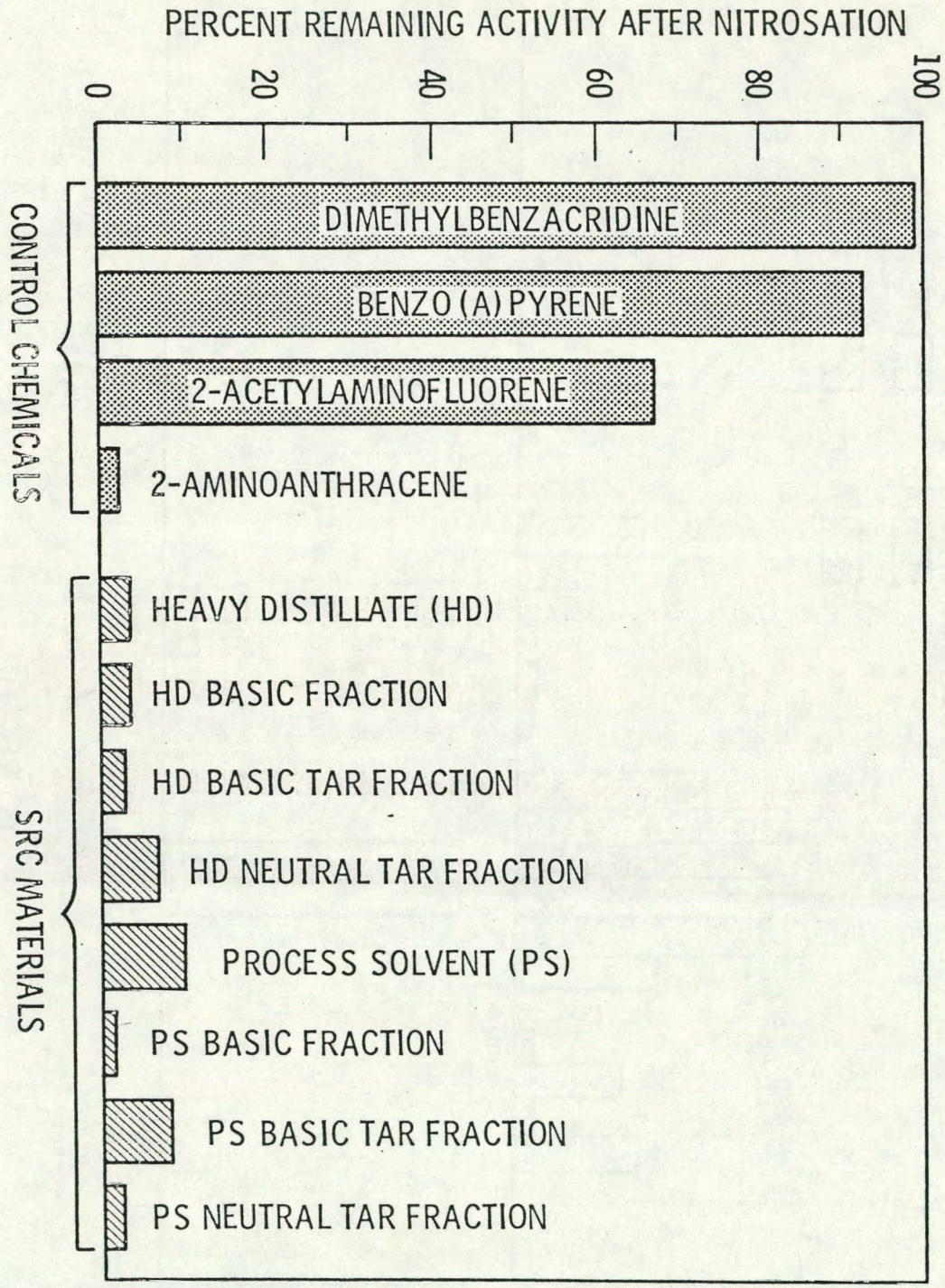

PERCENT REMAINING ACTIVITY AFTER NITROSATION
0
20
40
60
80
100
CONTROL CHEMICALS
DIMETHYLBENZACRIDINE
BENZO(A)PYRENE
2-ACETYLAMINOFLUORENE
2-AMINOANTHRACENE
SRC MATERIALS
HEAVY DISTILLATE (HD)
HD BASIC FRACTION
HD BASIC TAR FRACTION
HD NEUTRAL TAR FRACTION
PROCESS SOLVENT (PS)
PS BASIC FRACTION
PS BASIC TAR FRACTION
PS NEUTRAL TAR FRACTION

Figure 5

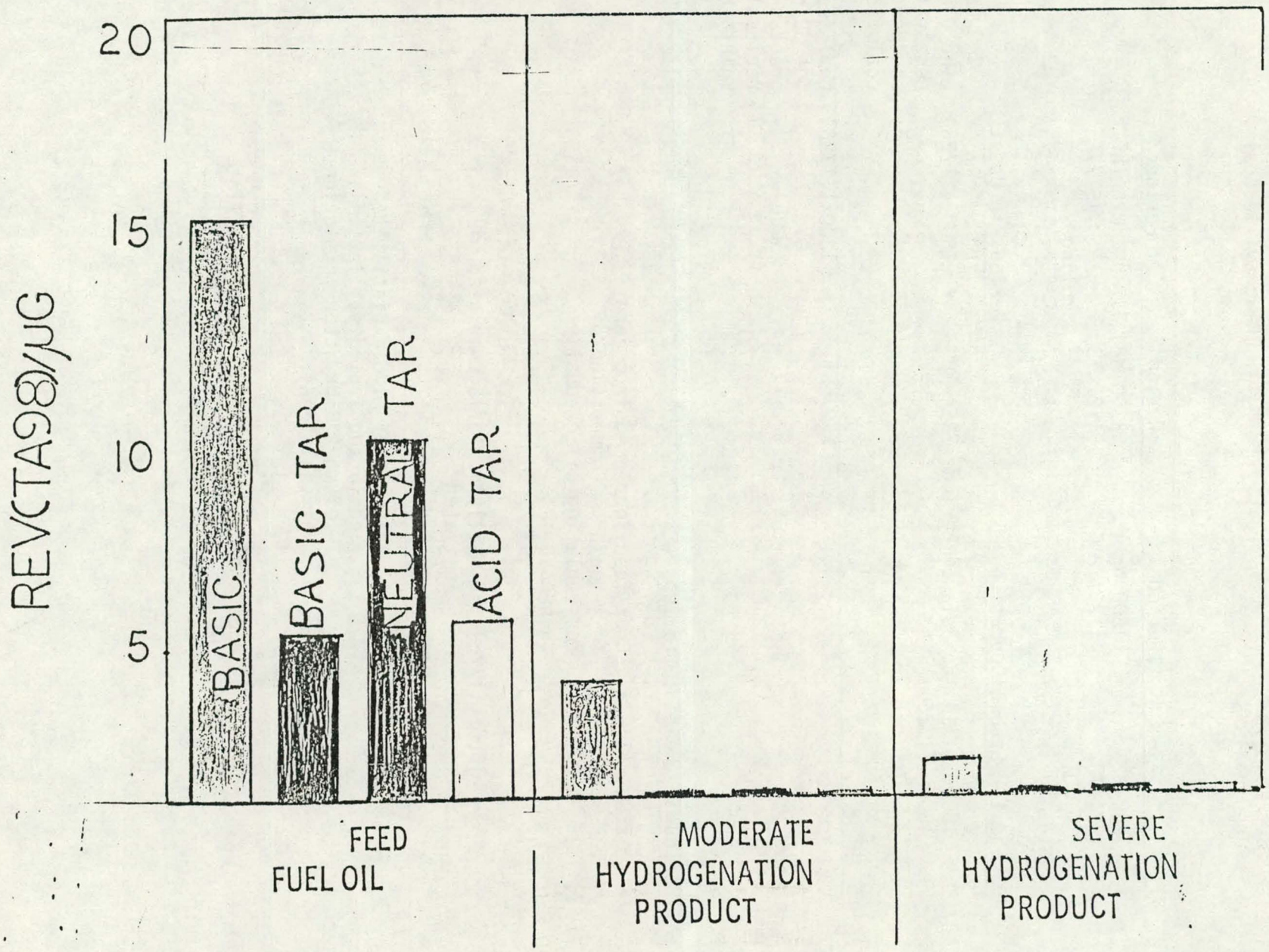

REVCTA98/µG
20
15
10
5
BASIC
BASIC TAR
NEUTRAL TAR
ACID TAR
FEED
FUEL OIL
MODERATE
HYDROGENATION
PRODUCT
SEVERE
HYDROGENATION
PRODUCT

Figure 6

HEAVY AND MIDDLE DISTILLATE SAMPLES

Run 78SR-18
January 1978

Blacksville Coal

	Period 1	Period 2	Period 3
1800 hr 1/24/78	1800 hr 1/26/78	2300 hr 1/27/78	0200 hr 1/31/78 sampling

Period 1: Stable Operation

- 1800 (1/24) to Balance Period, 78Sr-18
  1800 (1/26)

Period 2: Upset

- 1800 (1/26) Line plugging necessitated shutdown of coal feed.
- 1800 (1/26) to 2300 Coal feed out for 30 hr; process solvent recirculation maintained.

Period 3: Reactor Startup

- 2300 (1/27) Coal feed increased from zero to 1 ton/hr: Composition of recirculating oil gradually changes.
- 1600 (1/28) Distillation of accumulated oil initiated.
- 0430 (1/29) Sample of middle distillate collected from distillation column (30 hr after initiation of coal feed).
- 0500 (1/29) Sample of heavy distillate collected from distillation column.