United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

EPA-600/9-81-009
March 1981

Research and Development

EPA

A Matrix Approach to Biological Investigation of Synthetic Fuels

EPA 600/9-81-009
March 1981

A MATRIX APPROACH TO BIOLOGICAL INVESTIGATION OF SYNTHETIC FUELS

Proceedings of a Conference Cosponsored by
the U.S. Environmental Protection Agency and Oak Ridge National Laboratory
through the EPA/DOE Fossil Fuels Research Materials Facility

Research Triangle Park, North Carolina
April 26, 1979

Project Officer

David L. Coffin
Research Advisory and Special Studies Office
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

Documentation is provided for a conference cosponsored by the U.S. Environmental Protection Agency and Oak Ridge National Laboratory and held in Research Triangle Park, North Carolina on April 26, 1979. The general topic is toxicological assessment of health effects from the rapidly developing synthetic fuels industry.

In particular, the discussions focus on the Paraho crude shale oil that was produced by Development Engineering, Inc. (Anvil Points, Colorado) and refined into diesel and jet fuels by the Standard Oil Company of Ohio. Summaries of both operations are presented. Also discussed is the collection, storage, and distribution to toxicologists of sample materials from these operations by the U.S. Environmental Protection Agency/U.S. Department of Energy Fossil Fuels Research Materials Facility (Oak Ridge National Laboratory).

Other chapters survey ongoing and planned testing of the Paraho shale oil materials by investigators from Oak Ridge National Laboratory, Battelle Pacific Northwest Laboratories, Lawrence Livermore Laboratory, and the U.S. Environmental Protection Agency. The application of microbial, cellular, and whole-animal bioassays is considered.

CONTENTS

ABBREVIATIONS

API	-- American Petroleum Institute
aprt	-- adenine phosphoribosyltransferase
ATPase	-- adenosine-triphosphatase
BaP	-- benzo(a)pyrene
CHO	-- Chinese Hamster ovary
DFM	-- Diesel Fuel Marine
DOE	-- Department of Energy
DNA	-- deoxyribonucleic acid
EMS	-- ethyl methane sulfonate
EO	-- equivalent oil
EPA	-- Environmental Protection Agency
ESB	-- ether-soluble base
GC/MS	-- gas chromatography/mass spectrometry
hgprt	-- hypoxanthine-guanine phosphoribosyltransferase
hprt	-- hypoxanthine phosphoribosyltransferase
i.d.	-- inner diameter
JP	-- Jet Propellant
LD_{50}	-- median lethal dose
LLL	-- Lawrence Livermore Laboratory
ONR	-- Office of Naval Research
ORNL	-- Oak Ridge National Laboratory
PAH	-- polycyclic aromatic hydrocarbon
RNA	-- ribonucleic acid
SCE	-- sister chromatid exchange
SOHIO	-- Standard Oil Company of Ohio
tk	-- thymidine kinase
TLC	-- thin layer chromatography

1. OVERVIEW OF THE FOSSIL FUELS RESEARCH MATERIALS FACILITY

D. L. Coffin

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

INTRODUCTION

As everyone knows, there is a shortage of energy, and there will certainly be a place for alternate synthetic fuels derived from either coal or shale. Accordingly, there is a great need for rapid application of toxicology in this developing industry. The U.S. Environmental Protection Agency (EPA)/U.S. Department of Energy (DOE) Fossil Fuels Research Materials Facility ("Repository") was created to involve toxicologists "on the ground floor" in the industry, before appreciable human exposure occurs through environmental problems, in plants, or with users. EPA's hope was that, with the synthetic fuels industry, we will be "ahead of the game" for a change.

A substantial amount of research must be accomplished, and accomplished quickly, if it is to have any impact. Therefore, EPA welcomes and encourages the participation of all interested toxicologists; the Repository was created as a focal point for such collaboration. In the beginning, we participated in numerous discussions with Lt. Comdr. Leigh Doptis of the U.S. Navy, and we became especially cognizant of the Navy's needs.

THE MATRIX APPROACH

The term "repository" should not imply a place where chemicals are merely "put in a closet." The primary function of the Repository is to provide a way of moving from the technologies to the biologists and back to the technologies, so that the biologists work with materials relevant to the technologies, that they communicate with one another, and that they have unified specimens to permit nearly automatic comparison of one biological technique to another. This matrix approach is important from the standpoint of such developing models as the Ames assay, because — despite all the ongoing work — we still lack close comparison between these methods and some of the older, more classical methods of skin bioassay, etc. The Repository automatically encourages comparison of this sort, because a matrix of investigators applies different techniques to identical materials. The function of the Repository is not only to provide the samples, but also to aid in communication between the investigators and the technologists and administrative personnel.

HISTORY

The Search for Samples

A fundamental goal in creating the Repository was to encourage toxicological studies of synthetic fuel materials before there was large capital investment and commercial development. We wanted to become involved at a point somewhere between the developmental stage (the "bench model" level) and the commercial drive. We were interested in enterprises of sufficient size to make testing practical and to provide enough material for complete analysis. In the case of shale oil, for example, we envisioned an analysis that would extend from mining all the way through end use of the product, to provide input on environmental problems (with mining and crushing), worker health problems (retorting, refining, distribution, and use), and problems associated with end use (usually, some sort of combustion).

At the beginning, it was difficult to find technologies that would lend themselves to this type of analysis. We found, in certain instances, that a technology had become outdated and was therefore unsuited for our purposes. Alternately, many of the governmental efforts remained at a "bench model" stage and were therefore equally unsuited to our desired approach (i.e., a complete analysis).

The Paraho Operation

In view of this situation, the Repository team became quite excited at the opportunity to examine the recent U.S. Navy/Paraho operation. Paraho represented a chance to obtain sufficient materials for biological testing: production of $\sim 10^5$ bbl (from our standpoint, a significant quantity) of crude shale oil was planned. Because the Paraho operation was the best available approximation of an initial commercial module, then, we concentrated most of our resources on it. This, we felt, was a sensible approach: the Navy had identified shale oil as the closest in time to a usable fluid fuel of any of the alternate sources, and Paraho was (and is) the largest shale oil development effort available to us.

To date, the Paraho operation has progressed all the way through the stage of refining. The products are in the Repository, ready for distribution. Materials from mining and retorting are already distributed.

OUTLOOK

Subsequent chapters in this volume lay out the plan that has been developed among individual investigators, show how the efforts of these investigators interrelate, and indicate how data will be returned to the technologies. The whole point of the effort, of course, is to help technologies develop clean, safe fuels. If certain problems cannot be removed, the goal is to develop methods of occupational hygiene that will prevent injury to workers.

The scope of this matrix approach extends far beyond the range of EPA's immediate interests: it bears not only on environmental factors and problems of the community at large, but also on worker health and product safety. (For example, we are cooperating with the program of Comdr. Lawrence J. Jenkins of the Naval Medical Research Institute at Wright-Patterson Air Force Base.) The Repository will attempt to cover the entire field; the methods and lines of communication developed for the Paraho operation will serve as a model for investigation of other fuels.

Historically, the perspective of industrial toxicology has been applied only after some sort of problem (e.g., illness in workers) has developed. EPA sees the developing synthetic fuels industry as an unusual and important opportunity — indeed, as a challenge — for toxicologists to become involved "on the ground floor" in assessing potential hazard. Hopefully, the coordinating efforts of the EPA/DOE Fossil Fuels Research Materials Facility will help to efficiently and successfully meet this challenge.

2. DISTRIBUTION OF PARAHO OIL SHALE AND SOHIO-REFINED PARAHO SHALE OIL MATERIALS

W. H. Griest
M. R. Guerin

Analytical Chemistry Division
Oak Ridge National Laboratory
Post Office Box X
Oak Ridge, Tennessee 37830

INTRODUCTION

The function of the U.S. Environmental Protection Agency (EPA)/U.S. Department of Energy (DOE) Fossil Fuels Research Materials Facility ("Repository") is to obtain, catalog, store, and distribute research materials from synthetic fuels production to qualified health effects investigators. An additional function is to provide physical/chemical fractionation and characterization of high-priority materials for health effects testing. Data from the study of these materials are returned to the source of the samples. In the case of the oil shale industry, materials corresponding to both production and refining of shale oil are now available from the Repository. This paper briefly summarizes the collection, cataloging, and distribution of these materials.

COLLECTION OF SAMPLES

Samples from the retorting of oil shale were collected by a Repository subcontractor at the Paraho above-ground retorting process demonstration site at Anvil Points, Colorado, in the fall of 1977. At that time, a 10^5-bbl production run for the U.S. Navy was in progress. Itemized in Table 2-1, these

TABLE 2-1. REPOSITORY INVENTORY OF OIL SHALE MATERIALS FROM PARAHO ABOVE-GROUND RETORT

Repository Sample No.	Date Received	Description	Quantity
4204	11-8-77	Raw Shale	50 gal
4206	11-4-77	Airborne Raw Shale (hi-vol)	21 g
4209	11-8-77	Raw Shale Particles from Baghouse	5 gal
4205	11-8-77	Retorted Shale	25 gal
4207	11-8-77	Airborne Retorted Shale (hi-vol)	10 g
4208	11-8-77	Retorted Shale Particles from Baghouse (collected from screw conveyor)	5 gal
		Retorted Shale Particles from Baghouse	
4211	8-1-78[a]	(a) 0-10 μm sized fraction	45 lb
4212	8-1-78[a]	(b) >10 μm sized fraction	350 lb
4213	11-8-77	(c) unsized	40 lb
4203	11-8-77	Product Oil	8 gal
4202	11-8-77	Product Water (oil separation)	2 gal
4201	11-8-77	Process Water (gas line drain)	2 gal
4210	11-4-77	Thermo-Oxidizer Stack Particles	50 mg

[a]Date of receipt of sized particle fractions from subcontractor.

collected materials correspond to both bulk and airborne samples of raw and retorted oil shale. The latter has been sized into respirable and nonrespirable particle fractions. Samples of the shale oil and of process and product water also are available.

In late 1978 and early 1979, the Paraho shale oil was refined into diesel and jet fuels by the Standard Oil Company of Ohio (SOHIO) at its Toledo, Ohio refinery. SOHIO personnel collected samples of the raw and hydrotreated shale oils, intermediate materials, and final, finished products in 55-gal stainless steel drums. The samples were shipped to the Repository by mid-March, 1979. Table 2-2 lists the Repository inventory of these materials.

Corresponding petroleum-derived jet and diesel fuel products (Table 2-3) also were obtained directly from Comdr. L. J. Jenkins of Wright-Patterson Air

TABLE 2-2. REPOSITORY INVENTORY OF SOHIO-REFINED AND -SUPPLIED PARAHO SHALE OIL MATERIALS

Repository Sample No.	Date of Sampling	Description	SOHIO Reference No.	Quantity
4601	11-03-76	Crude Shale Oil	CSO-556	55 gal
4602	11-29-78	Hydrotreated Shale Oil	C5HTSO-554	55 gal
4603	11-21-78	Weathered Gas Feedstock	WGFS-55	5 gal
4604	11-22-78	JP-5 Before Treating (Precursor)	PRE JP5-555	55 gal
4605	11-27-78	JP-8 Before Treating (Precursor)	PRE JP8-555	55 gal
4606	11-21-78	DFM Before Treating (Precursor)	PRE-DFM-555	55 gal
4607	11-30-78	Hydrotreated Residue	HTR-555	55 gal
4608	1-24-79	JP-5 Product	FIN JP5-554	55 gal
4609	11-27-78	JP-8 Product	FIN JP8-554	55 gal
4610	2-21-79	DFM Product	FIN DFM	55 gal
4611	11-28-78	JP-5 Product	FIN JP5-N-2A	1 gal
4612	not supplied	Acid Sludge from DFM Treatment	AS-52	5 gal

TABLE 2-3. REPOSITORY INVENTORY OF PETROLEUM-DERIVED JET FUELS AND DFM[a]

Repository Sample No.	Date Received	Material	Quantity
4613	1-19-79	JP-4 Product	55 gal
4614	1-10-79	JP-5 Product	55 gal
4615	1-10-79	JP-8 Product	55 gal
4616	1-10-79	DFM Product	5 gal

[a]Materials obtained from Comdr. L. J. Jenkins, Wright-Patterson Air Force Base, Ohio.

Force Base, Ohio, in early January of 1979. These fuels are "reference" materials which the Navy is using for comparison with shale-oil-derived fuels, and they were obtained to allow investigators working with SOHIO-refined Paraho shale oil materials to use the same "references."

DISTRIBUTION OF SAMPLES

In early May 1979, the SOHIO-refined Paraho shale oil materials and Wright-Patterson Air Force Base petroleum equivalents were carefully mixed and aliquoted into clean (methanol-rinsed, dried, and rinsed with sample itself) amber borosilicate bottles, except where large sample volumes required other containers. After the headspace was briefly flushed with argon, each container was sealed with a Teflon-lined cap and labeled. Shipments to the investigators were made in late May 1979. Table 2-4 shows the distribution of the materials. Table 2-5 lists the names, addresses, and phone numbers of all investigators.

Requests for additional samples should be directed to L. B. Yeatts (FTS 624-4863; commercial 615-574-4863) or W. H. Griest (FTS 624-4868; commercial 615-574-4868).

RETURN OF DATA

Data returned by the investigators will be compiled and fowarded to the Navy and other sponsors. SOHIO and Development Engineering, Inc. (Paraho) have requested that all investigators allow them to review any papers before publication; this review can be arranged through the Repository.

STORAGE IN THE REPOSITORY

Bulk quantities of the remaining materials are being stored under ambient conditions in the stainless steel drums until refrigerated storage can be arranged. Smaller aliquots are being refrigerated in borosilicate glass for future requests and for stability studies. Determinations of infrared spectrum, viscosity, simulated distillation, elemental composition, and major

TABLE 2-4. REPOSITORY DISTRIBUTION OF SOHIO-REFINED PARAHO SHALE OIL MATERIALS AND PETROLEUM EQUIVALENTS

Material	Investigator No.: (see Table 2-5)	1	2	3	4	5	6	7	8	9	10	11	12	13
		Sample Requirements												
	Study:	Carcinogenesis	PAH Analysis	Comprehensive Analysis	Marine Ecosystem	Carcinogenesis	Diesel Exhaust	Mutagenesis	Mutagenesis & Chemical Analysis	Mutagenesis & Short-term Animal	Pond Ecosystem	Gas Chromatography	Acute Oral Mouse Toxicity	Drosophila Mutagenesis
Shale Oil														
Crude Shale Oil (4601)		2 liters	500 ml	4 liters		4 liters		100 ml	10 liters	100 ml			100 ml	100 ml
Hydrotreated Shale Oil (4602)		2 liters	500 ml	4 liters		4 liters		100 ml	10 liters	100 ml			100 ml	100 ml
Weathered Gas Feedstock (4603)				4 liters				100 ml						100 ml
JP-5 Precursor (4604)				4 liters		4 liters		100 ml						100 ml
JP-8 Precursor (4605)				4 liters				100 ml						100 ml
DFM Precursor (4606)		2 liters	500 ml	4 liters		4 liters		100 ml						100 ml
Hydrotreated Residue (4607)		2 liters	500 ml	4 liters		4 liters		100 ml	10 liters		1 liter		100 ml	100 ml
JP-5 Product (4608)				4 liters		4 liters		100 ml	10 liters			50 ml		100 ml
JP-8 Product (4609)				4 liters		4 liters		100 ml	10 liters					100 ml
DFM Product (4610)		2 liters	500 ml	4 liters	32 liters	4 liters	20 liters	100 ml	10 liters					100 ml
Acid Sludge (4612)				1 liter				100 ml						100 ml
Petroleum Equivalent														
JP-5 Product (4614)						4 liters		100 ml	10 liters					100 ml
JP-8 Product (4615)						4 liters		100 ml	10 liters					100 ml
DFM Product (4616)		500 ml			100 ml	4 liters		100 ml	100 ml					100 ml

TABLE 2-5. INVESTIGATORS WHO HAVE RECEIVED SOHIO-REFINED PARAHO SHALE OIL MATERIALS AND PETROLEUM EQUIVALENTS

Investigator No. (see Table 2-4)	Investigator, Sponsor	Phone	Address
1	Dr. William Barkley (API)	513-872-5785	Department of Environmental Health Kettering Laboratory University of Cincinnati Medical Center 3223 Eden Avenue Cincinnati, Ohio 45219
2	S. C. Blum (API)	201-474-3303	Exxon Research & Engineering Company Analytical & Information Division Post Office Box 121 Linden, New Jersey 07036
3	L. W. Burdett (API)	714-528-7201	Union Oil Company of California Union Research Center Post Office Box 76 Brea, California 92621
4	Dr. Norman Richards (EPA)	FTS-686-9011	U.S. Environmental Protection Agency Environmental Sciences Research Laboratory Sabine Island Gulf Breeze, Florida 32561
5	Dr. Mike Holland (DOE)	FTS-624-0678	Biology Division Oak Ridge National Laboratory Post Office Box X Oak Ridge, Tennessee 37830
6	Dr. David Coffin (Dr. Ronald Bradow) (EPA)	FTS-629-2585	U.S. Environmental Protection Agency Health Effects Research Laboratory Research Triangle Park, NC 27711
7	Dr. J. L. Epler (DOE)	FTS-624-0841	Biology Division Oak Ridge National Laboratory Post Office Box X Oak Ridge, Tennessee 37830
8	Dr. L. M. Holland (DOE)	FTS-843-2747	Los Alamos Scientific Laboratory c/o Receiving Department, SM-30 Los Alamos, New Mexico 87545
9	Dr. F. T. Hatch (DOE)	FTS-532-5611	Lawrence Livermore Laboratory Post Office Box 5507 Livermore, California 94550
10	Dr. J. M. Giddings (DOE)	FTS-624-7337	Environmental Sciences Division Oak Ridge National Laboratory Post Office Box X Oak Ridge, Tennessee 37830
11	Comdr. M. J. Cowan (U.S. Navy)	FTS-775-3116	Naval Medical Research Institute Toxicology Detachment NMRI/TD Wright-Patterson Air Force Base Dayton, Ohio 45433
12	Dr. H. F. Witschi (DOE)	FTS-624-0801	Biology Division Oak Ridge National Laboratory Post Office Box X Oak Ridge, Tennessee 37830
13	Dr. S. Zimmering (DOE)	401-863-2620	Division of Biology and Medicine Brown University Providence, Rhode Island 02912

organics (gas chromatographic profile) will be conducted periodically on samples stored in flint glass, borosilicate glass, and stainless steel under ambient and refrigerated conditions.

ACKNOWLEDGMENTS

This research was sponsored by the U.S. Environmental Protection Agency under Interagency Agreement IAG D-7-0129, under Union Carbide Corporation contract W-7405-ENG-26 with the U.S. Department of Energy.

3. RECENT PARAHO OPERATIONS

R. N. Heistand
J. B. Jones, Jr.

Development Engineering, Inc.
Post Office Box A, Anvil Points
Rifle, Colorado 81650

INTRODUCTION

The Paraho Process

The Paraho process is operated at Anvil Points, Colorado by Development Engineering, Inc., a subsidiary of Paraho Development Corporation. The process and retort have been described previously (Pforzheimer 1974).

The retort is a cylindrical, vertical kiln having a refractory-lined carbon steel shell (Figure 3-1). Near the top of the retort is the off-gas collector, where the oil mist and gas are removed from the retort. Below the off-gas collector are three gas/air distributors located at separate levels in the retort. The bottom gas/air distributor is located in the grate mechanism at the bottom of the retort. This grate at the bottom and the rotating spreader at the top are the only moving pieces within the retort.

The Paraho retort can be operated in several modes. Figure 3-2 shows the Direct Mode, where combustion required to produce the heat for retorting occurs within the retort. Raw shale enters the top of the retort and is preheated in the mist formation zone by the gases carrying the oil mist out of the retort. The preheated shale next enters the retorting zone, where hot gases rising

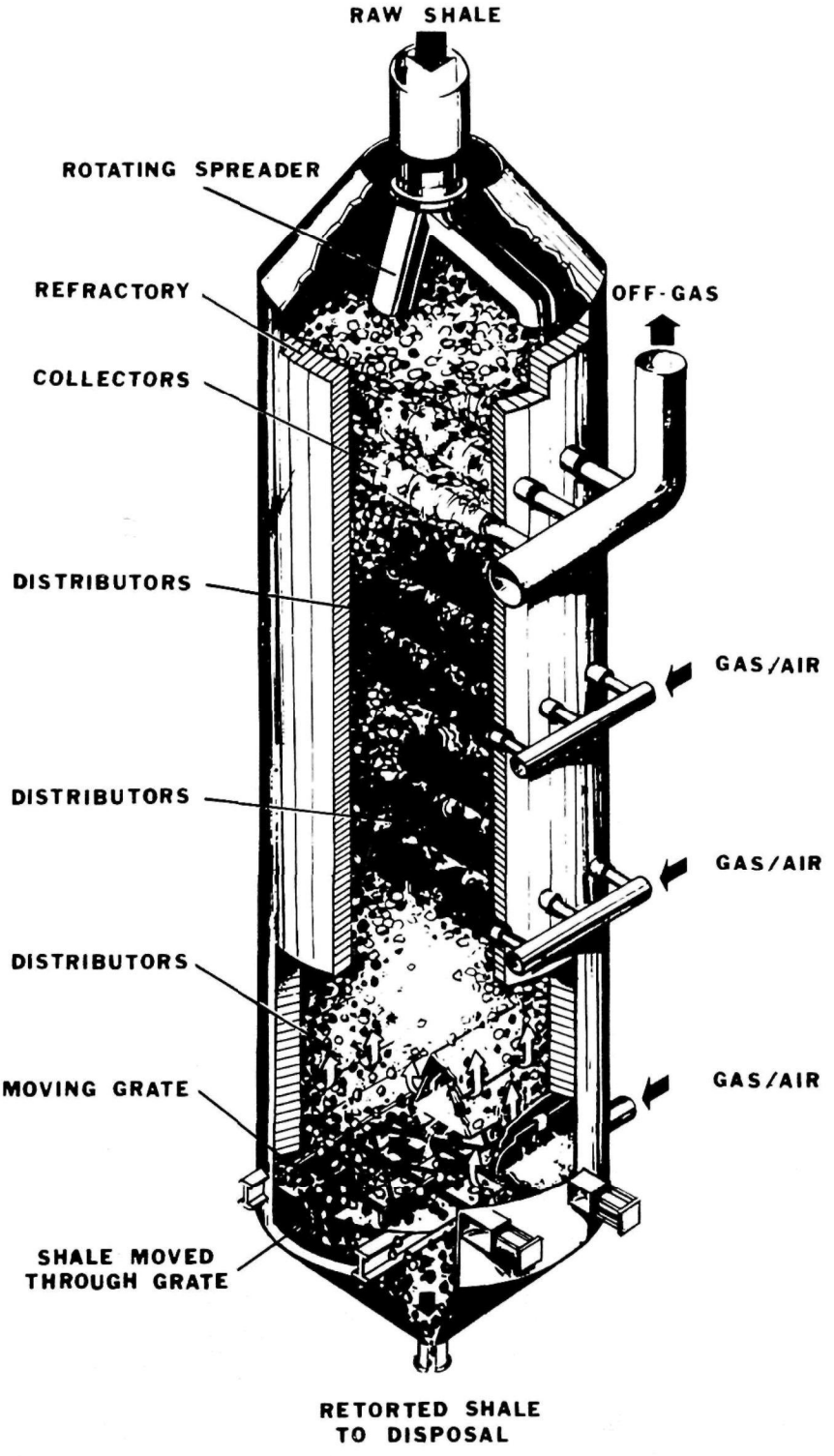

Figure 3-1. Paraho retort.

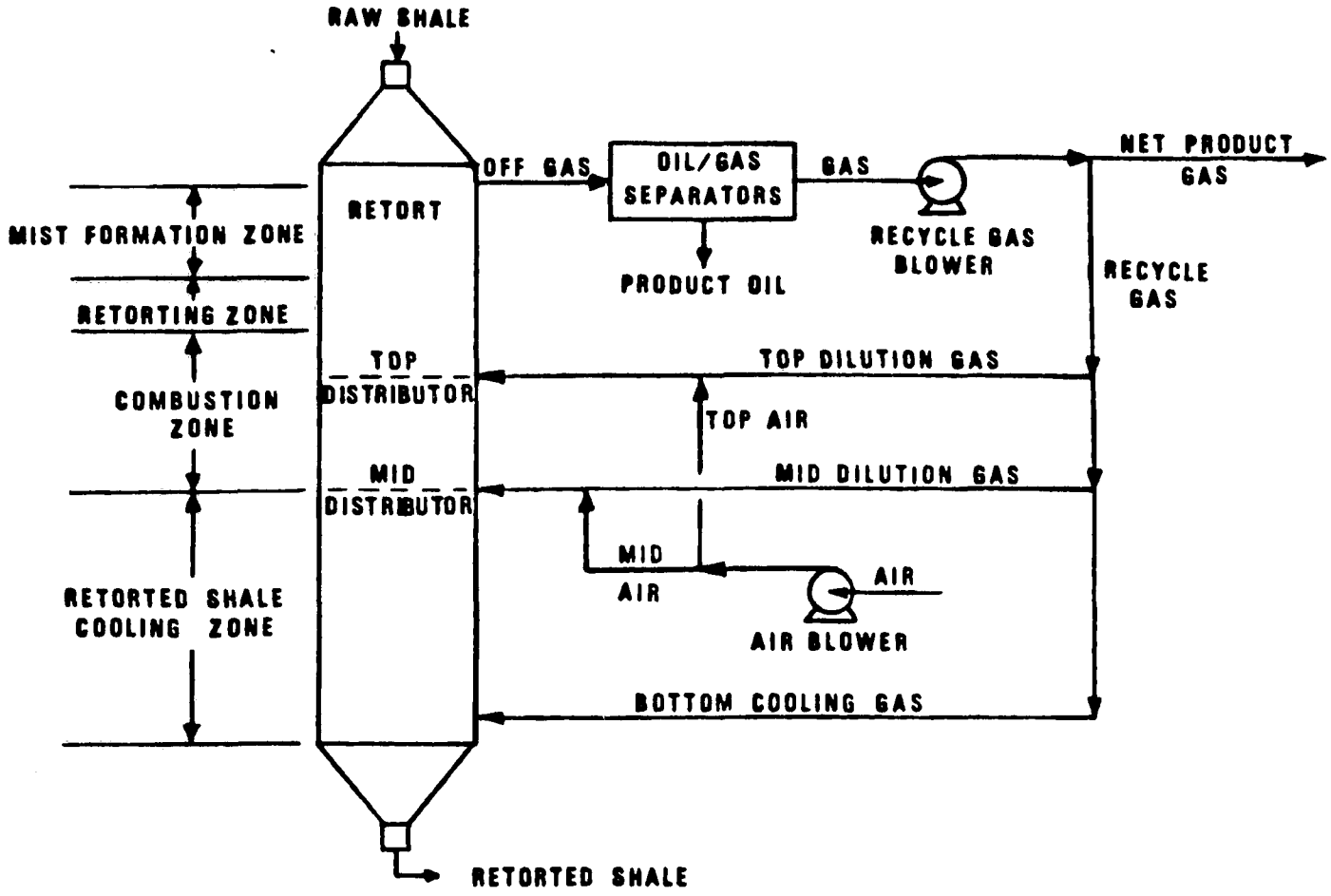

Figure 3-2. Direct Mode schematic.

from the internal combustion heat the shale to retorting temperature. Upon retorting, the solid organic kerogen in the shale breaks down to gas, oil, and coke. The gas and oil are swept upward with the hot gases, and the coke remains on the retorted shale. As the retorted shale enters the combustion zone, some of this coke is burned with the air introduced through the gas/air distributor. Only enough air is introduced to produce sufficient heat for the retorting process. Recycle gas is added with the air to ensure even distribution across the bed and to control the flame temperature. Below the middle distributor, the retorted shale enters the cooling zone, where it is cooled by recycle gas rising from the bottom distributor. What the Paraho retort involves, then, are countercurrent flows: gases rise while shale moves downward under the force of gravity and as controlled by the grate. These flows produce hot combustion and retorting zones in the middle with heat exchange at both top and bottom, so that both gases (and oil mist) and retorted shale are relatively cool upon leaving the retort.

Another mode of operation of the Paraho retort is the Indirect Mode. In this case, the air blower is replaced by a heater, and the recycle gas entering the top and middle distributors is heated externally. No combustion occurs within the retort; the product gas is not diluted with N_2 and carbon dioxide (CO_2) by-products of combustion, and the coke remaining on the retorted shale is not utilized.

Brief History

Paraho oil shale retorting operations began with the Paraho Oil Shale Demonstration. That demonstration, a $10-million, 3-year project involving 17 industrial participants, is described elsewhere (Jones 1976, 1977). One of the project achievements was the refining of $\sim 10^4$ bbl of crude shale oil into military fuels.

Based in part upon the achievement of that refining run, the U.S. Office of Naval Research (ONR), U.S. Department of Navy, and U.S. Department of Energy

(DOE) contracted with Paraho to continue research and development of surface retorting technology. These contracts consisted of the following:

- refurbishment of the facility for limited operation;
- installation of crude shale oil storage tanks;
- continued research and development, including production of up to 10^5 bbl of shale oil.

Operations were carried out under ONR contracts until December 31, 1977 and were continued under a DOE contract into 1978.

During the third quarter of 1978, the crude shale oil produced during these recent operations (1977-78) was shipped for refining into military fuels. The shipment was carried out by rail, using a shuttle of 40 jumbo rail tank cars. This was the largest shipment of crude shale oil in the United States to date. Refining was carried out at the Toledo refinery of the Standard Oil Company of Ohio.

During these recent Paraho operations (especially during the last half of 1977), many researchers and specialists visited the Anvil Points site to perform environmental monitoring and to obtain samples for further research. Samples for the U.S. Environmental Protection Agency (EPA)/DOE Fossil Fuels Research Materials Facility were taken during this period.

RECENT PARAHO OPERATIONS

Mining

During the recent operations, the Anvil Points mine utilized equipment that had been employed by Paraho in previous operations. This equipment consisted of a rotary drill jumbo, a mechanical scaler, a roofbolter, a 5-yd^3 wheel loader, 50-t haul trucks, a water truck, a grader, air compressors, and ventilating fans. During the 1977-78 operations, nearly 2 x 10^5 t of shale were mined for use by DOE and in the Paraho project. Figure 3-3 illustrates the advances of the room-and-pillar mine during these recent operations.

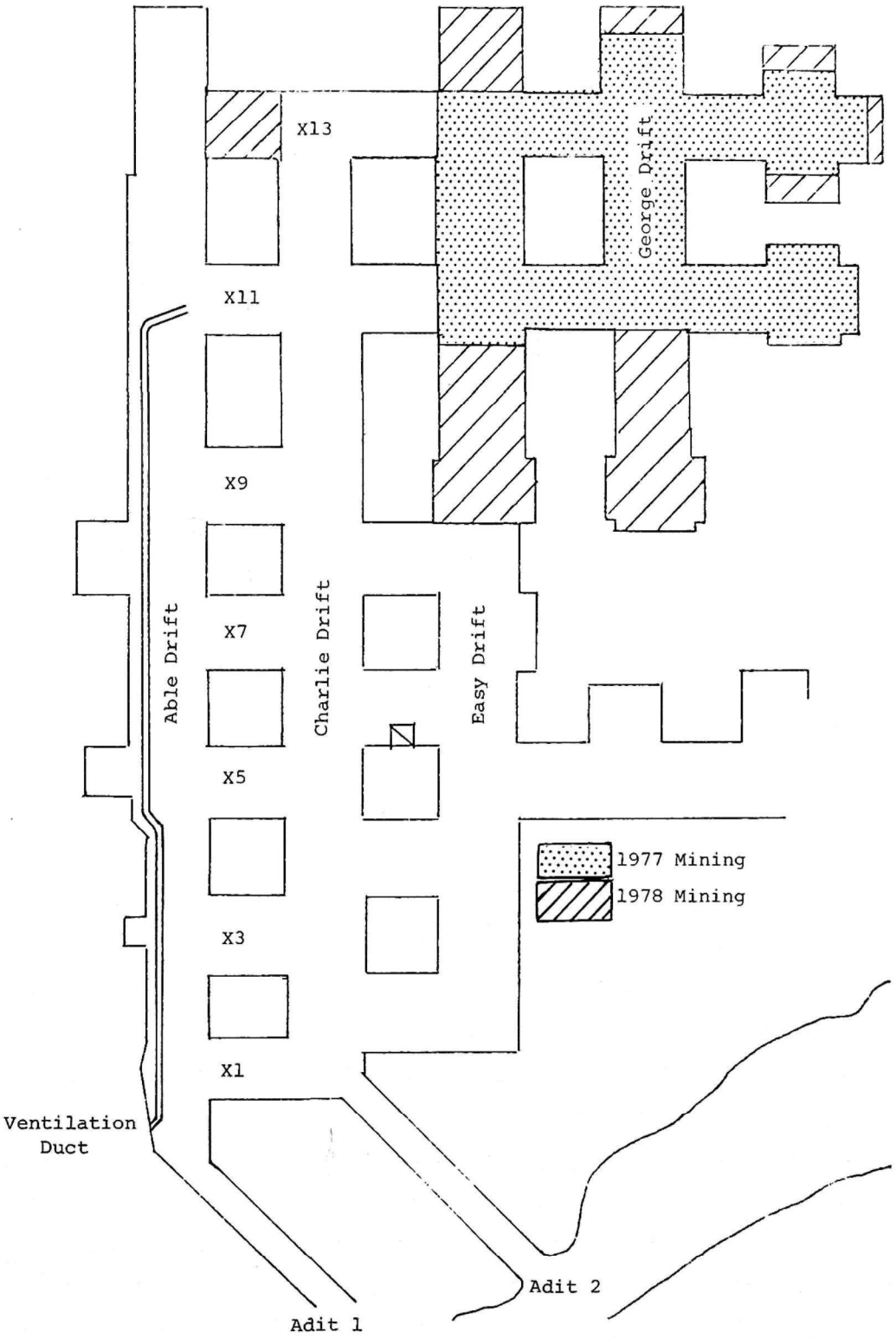

Figure 3-3. Anvil Points mine.

Crushing

The crushing operations used a series of crushers provided by the Navy. These consisted of a primary jaw crusher, a secondary jaw crusher, and a tertiary double roll crusher. Other support equipment included a small loader, storage bins, and screens.

Retort Production

Table 3-1 presents a general overview of retort production during the recent Paraho operations. Data are from the 8.5-ft i.d. Semi-Works operations. Shale grade varied from 24.5 to 36.6 gal/t; shale rate varied from 416 to 536 $lb/h/ft^2$. Oil production varied from 140 to 200 bbl/d. This production was limited by the oil/gas separation equipment. When daily production exceeded 180 bbl/d, oil/gas separation equipment capacity was exceeded, and the oil was not completely separated from the recycle gas. Gas production averaged 1.5×10^6 $stdft^3/d$, with an average gross heating value of 150 $Btu/stdft^3$. Table 3-1 calculates equivalent oil (EO) of the gas on a basis of 6×10^6 Btu = 1.0 bbl of oil. On this basis, the gas EO production ranged from 30 to 50 bbl/d.

Products

Tables 3-2 through 3-4 outline properties of the raw shale and of typical products from the recent operations. Except for a low water content, the crude shale oil (Table 3-2) is typical of most crude shale oils. Water is removed from the product oil by simple decantation, in order to meet ONR specifications. The product gas (Table 3-3) is typical of Direct Mode operations — high in N_2 and CO_2. Hydrogen sulfide (H_2S) is ~0.3% by vol., or 3000 ppm. The raw and retorted shales (Table 3-4) are typical of Anvil Points operations. Note that most of the organic carbon present in the raw shale is removed. Most of the sulfur remains, however.

TABLE 3-1. SEMI-WORKS RETORT PRODUCTION[a]

	1977				1978	
	Jan	Apr	May	Dec	Jan	Sept
Shale						
Rate (lb/h/ft^2)	432	486	418	536	416	502
Grade (gal/t)	24.5	29.7	24.7	36.6	24.5	29.2
Product Gas						
Production (1000 stdft^3/d)	1400	1500	1300	1700	1400	1800
GHV (Btu/stdft^3)	118	141	118	153	120	180
Equivalent Oil (bbl/d)	30	35	30	40	35	50
Product Oil						
Production (bbl/d)	140	200	145	190	145	180

[a]From Jones and Heistand (1979).

TABLE 3-2. PROPERTIES OF CRUDE SHALE OIL

Parameter	Value
Gravity (°API)	21.4
Viscosity (SSU at 130°F)	83
Viscosity (SSU at 210°F)	48
Pour point (°F)	85
Water (% by wgt.)	0.3
Sediment (ml/100 g)	0.1
Carbon (% by wgt.)	84.8
Hydrogen (% by wgt.)	11.4
Nitrogen (% by wgt.)	2.0
Sulfur (% by wgt.)	0.6

TABLE 3-3. PROPERTIES OF PRODUCT GAS

Parameter	Value
Dry Gas (% by vol.)	
H_2	5.5
N_2	61.0
O_2	0
CO	2.9
CH_4	2.4
CO_2	24.2
C_2H_4	0.7
C_2H_6	0.6
C_3	0.6
C_4	0.6
C_5+	0.6
H_2S	0.3
NH_3	0.6
Total	100.0
Water Vapor (% by vol.)	17.5
Heating Value (Btu/stdft^3 (dry))	145

Operability, Reliability, and Yields

Operability of the Paraho Semi-Works retort was most encouraging. The on-stream factor, determined by dividing the on-stream hours by the total hours, exceeded 90% for the recent operations (90.8% for 1977 and 90.7% for 1978). These consistently high on-stream factors are a measure of the operational reliability of the Paraho above-ground retorting process.

Another measure of reliability is the long continuous run achieved during performance of the ONR contracts in the first half of 1977. Although long,

TABLE 3-4. PROPERTIES OF RAW AND RETORTED SHALE

Analysis	Parameter	Value: Raw Shale	Value: Retorted Shale
Fischer Assay	Oil (% by wgt.)	10.46	0.06
	Water (% by wgt.)	1.07	0.41
	Oil (gal/t)	27.39	0.16
	Gas + Loss (% by wgt.)	1.99	0.18
Chemical	Mineral CO_2 (% by wgt.)	17.54	15.63
	Ash (% by wgt.)	66.67	81.44
	Moisture (% by wgt.)	0.88	
Elemental	Carbon (% by wgt.)	16.65	6.48
	Hydrogen (% by wgt.)	1.75	0.18
	Nitrogen (% by wgt.)	0.52	0.23
	Sulfur (% by wgt.)	0.74	0.80

continuous operations were not a principal objective, a 105-d continuous run was achieved. The Semi-Works retort was successfully fired off on January 5, 1977 for the 10-d shakedown operations. Without shutdown, operations were continued. After the required 1.25×10^4 bbl of crude shale oil had been produced, research efforts were accelerated to determine maximum oil production. An oil production level of 200 bbl/d was achieved. This exceeded the limits of the oil/gas separation, and operations were shut down on April 20, 1977. Total production of shale oil meeting ONR specifications approached 1.5×10^4 bbl of dry oil. Lost time (downtime) during this continuous operation totaled 4.2 h, most of which was devoted to standby while the raw shale rotary seal was serviced. Other details of the 105-d continuous operation are presented elsewhere (Jones and Heistand 1979).

Retort yields are obtained by comparing retort performance with results of the Fischer Assay (Heistand 1976). The Fischer Assay is an empirical laboratory method used to determine the oil potential of shale. By comparing the energy products of the Paraho Direct Mode operations (oil, gas EO, plus

the thermal energy EO supplied as heat from the raw shale feed) with the products of the Fischer Assay (oil plus gas EO), a retort yield is obtained (Heistand 1979). Typical retort yields for Paraho Direct Mode operations average 114% of assay (Table 3-5).

The reliability of these data and of the data presented in the balance of this report is verified by good closures of material and elemental balances (Table 3-6). Balances for mean data of ~100% for each of the 2-year periods are typical of results from individual test days. Normally, the overall weight balance was 100 ± 1% and the balance for total carbon was 100 ± 4%. These good balance closures indicate accurate measurements of both process flows and product composition, and demonstrate that the data are valid and reliable.

RESEARCH

Research studies directed towards improving operational reliability, increasing oil yield and production, and minimizing environmental impacts were carried out during the 1977-78 operations. Major emphasis was given to retorting operations studies, which involved more than 100 24-h test days. These studies employed both the 2.5-ft i.d. Pilot Plant and the 8.5-ft i.d. Semi-Works. As noted previously, although both retorts were subjected to wide variations in operating conditions, the overall operability and yields remained high.

Three of the research studies merit additional discussion. These are: shale grade; product gas desulfurization; and introduction of air to the lower section of the retort. These studies have been conducive to improving oil production, minimizing environmental impacts, and improving operability.

Shale Grade

Eight 24-h test periods were carried out in the Pilot Plant over a 2-week period using raw shale feed of grades of 26.0, 27.9, 31.4, and 35.2 gal/t (Table 3-7). Oil production mirrored raw shale grade: production increased

TABLE 3-5. MEASURES OF YIELD

Analysis	Parameter	Value (gal/t)	Percent of Assay
Fischer Assay	Oil	27.4	
	Gas EO	2.2	
	Total	29.6	
Paraho Yield	Oil	24.4	89.1
	Gas EO	6.0	
	Total	30.4	103
Residual Carbon Fuel Used	EO	3.3	
	Overall (Oil + Gas + Fuel)	33.7	114

TABLE 3-6. TYPICAL MATERIAL AND ELEMENTAL BALANCES[a]

Parameter	1977 Recovery (%)	1978 Recovery (%)
Weight	99.8	99.9
Total Carbon	99.0	99.2
Total Sulfur	103.1	100.3

[a]From Jones and Heistand (1979).

TABLE 3-7. SHALE GRADE RESEARCH[a]

Date	Raw Shale (gal/t)	Oil (bbl/d)	Gas EO (bbl/d)	Total (bbl/d)
1/9-10	26.0	11.5	1.4	12.9
1/15-16	27.9	16.5	1.7	18.2
1/20-21	31.4	18.3	1.5	19.8
1/25-26	35.2	20.8	1.9	22.7

[a]From Jones and Heistand (1979).

with increasing grade of shale. Retorting operability was not affected by use of rich (35.2 gal/t) shale. In order to substantiate this operability with rich shale, another research study was scheduled for the Pilot Plant. Over a 2-week operation, six 24-h test days were run using rich (36.2 ± 0.2 gal/t) shale. Retorting operability was not affected, and yields remained high for the six test days.

Gas Desulfurization

A special off-gas collector, designed to be installed without modifying the middle distributor, was used to test sulfur removal. Results of this Pilot Plant research are shown in Table 3-8. Sampling of gas within the retort indicated that H_2S was removed from the gas as it passed through the lower bed. At the middle distributor, the H_2S content of the recycle gas was reduced to 0. As the fraction of product gas taken through the middle off-gas collector increased, H_2S in the combined product gas fell from 0.30% to 0.17% by vol. Individual samples of the middle product gas stream showed H_2S levels to be as little as 0.05% by vol. or ~0.3 lb H_2S/MM Btu. Removal of product gas from the middle of the retort did not adversely affect yields or oil production. Significant quantities of H_2S can be removed from the gas in the lower retort, reducing environmental impacts without affecting operability or product yields.

TABLE 3-8. GAS DESULFURIZATION RESEARCH[a]

Date	Product Gas: Normal ($stdft^3/min$)	Product Gas: Middle ($stdft^3/min$)	Product Gas: H_2S (% by vol.)	Product Production: Oil (bbl/d)	Product Production: Gas EO (bbl/d)	Product Production: Total (bbl/d)
3/11	162	0	0.30	14.4	2.2	16.6
3/18	76	86	0.22	14.8	1.5	16.3
3/21	36	132	0.17	12.5	1.4	13.9
3/23	41	142	0.19	13.9	2.1	16.0

[a]From Jones and Heistand (1979).

Addition of Air to Bottom Distributor

The effect of adding air to the bottom distributor was studied in the Semi-Works retort over a 4-week period. Six tests (one to four 24-h periods each) were carried out in which bottom air was varied from 0 to 97 $stdft^3/min$ (Table 3-9). During a 2-d test period (6/27-28), a blockage in the middle distributor prevented introduction of sufficient air. Oil production fell; normally, the retorting operations would have been shut down. Instead, air was introduced through the bottom distributor, oil production again reached 163 bbl/d, and operations were continued for an additional 2 weeks. Although this technique can be used to extend operations, it does reduce thermal efficiency. This ability to shift the air injection to various distributors without adversely affecting production or operability demonstrates the Paraho technology's good mechanical design and serves to complement its commercial feasibility. Good on-stream service factors are enhanced in two ways: First, operations are extended. Secondly, unscheduled shutdowns requiring extensive downtime are avoided.

TABLE 3-9. RESEARCH ON ADDITION OF AIR TO BOTTOM DISTRIBUTOR[a]

Date	Air Bottom Distributor ($stdft^3/min$)	Product Production Oil (bbl/d)	Gas EO (bbl/d)	Total (bbl/d)
6/13,15	0	160	36	196
6/18-21	50	162	35	197
6/27-28	0	151	37	188
6/30	60	161	36	197
7/2	88	158	33	191
7/3-6	97	164	32	196

[a]From Jones and Heistand (1979).

CONCLUSIONS

The results of Paraho operations at Anvil Points have been most encourgaging. Highlights of these results are:

- Production of $>10^5$ bbl of crude shale oil
- Continuous periods of operation of >100 d
- Maintenance of a service factor of >90% for a 2-year period
- Demonstration of the ability to retort rich (>35 gal/t) shale
- Demonstration of the ability to produce low-sulfur (<500 ppm H_2S) gas
- Demonstration of the ability to operate until scheduled turnaround

With successful completion of two research projects utilizing the Paraho retorts, we are ready to proceed towards our next objectives:

- Design, construction, and operation of a full-size Paraho module
- Encouragement of oil shale commercialization
- Maintenance of status as a technology-licensing company

ACKNOWLEDGMENTS

The work described in this paper was carried out at the U.S. Department of Energy's Anvil Points Oil Shale Research Facility located on the Naval Oil Shale Reserves near Rifle, Colorado.

REFERENCES

Heistand, R. N. 1976. The Fisher Assay: Standard for the oil shale industry. Energy Sources 2(4):40.

Heistand, R. N. 1979. Product yields. 12th Oil Shale Symposium, Colorado School of Mines, Golden, Colorado, April 18-20.

Jones, J. B., Jr. 1976. The Paraho oil shale retort. 9th Oil Shale Symposium, Colorado School of Mines, Golden, Colorado.

Jones, J. B., Jr. 1977. Technical evaluation of the Paraho process. Eleventh Israel Conference on Mechanical Engineering, Technicon, Haifa, Israel.

Jones, J. B., Jr., and R. N. Heistand. 1979. Recent Paraho operations. 12th Oil Shale Symposium, Colorado School of Mines, Golden, Colorado, April 18-20.

Pforzheimer, H., Jr. 1974. Paraho — New prospects for oil shale. Chem. Eng. Prog. 70(9):62.

4. REFINING OF SHALE OIL BY SOHIO

D. L. Cawein

The Standard Oil Company of Ohio
Post Office Box 696
Toledo, Ohio 43694

INTRODUCTION

Some speakers at this conference have mentioned "energy shortage." There is no energy shortage; there are tremendous amounts of energy in the forms of oil shale, tar sand, coal, solar radiation, and so on. The problem is one of economics — there is not the low-cost energy that we had the luxury of using in the past.

The Standard Oil Company of Ohio (SOHIO) has been interested in oil shale as an alternate energy source for some 20 years. At the beginning, we acquired a reserve position in oil shale and entered into a joint venture to investigate what appeared to be a very likely first process that could reach an early stage of commercialization and be economic. After spending, with others, some 15 to 20 million dollars in the middle 1960's, we recognized that the technology was relatively close. There were technological problems, but they were solvable; the primary problem was the economics of the process.

Following this initial investigation, SOHIO continued to expand its studies and reserve position. At that time, the internal combustion vertical-shaft kiln that the U.S. Bureau of Mines had first researched many years ago was being further developed by Development Engineering, Inc. in a consortium called Paraho. To SOHIO, this appeared the most likely first entry into a

commercial oil shale industry. So SOHIO, along with Development Engineering, Inc., organized a consortium of 17 companies to further the research and development of that technology.

Paraho really spearheaded the operation. Paraho leased the plant from the U.S. Navy, worked with the Navy for continued funding (along with funding from various commercial oil companies, including SOHIO), and won approval for the 2-year operation to make 10^5 bbl of raw shale oil. Having made the raw shale oil, the next step was to convert it to products needed in Navy operations. Because of our interest in furthering the oil shale industry, SOHIO submitted to Paraho and the Navy a bid to refine 10^5 bbl into products similar to those the Navy was using. SOHIO proposed a two-step plan: (1) a laboratory pilot program, to assess the feasibility of the process; and (2) processing of the 10^5 bbl in commercial equipment. In summary, both steps were successful. At SOHIO's Research and Development Laboratory in Cleveland, we were able to process the raw shale oil into fractions very similar to products from crude oil. Secondly, in commercial equipment in our refinery, we were able to very nearly duplicate the laboratory results. This accomplishment, we believe, has important implications for future efforts. This confirmation of pilot plant results increases the confidence in future pilot studies.

SOHIO'S TOLEDO REFINERY

The SOHIO refinery in Toledo, Ohio is a medium-sized oil refinery: 1.2×10^5 bbl/d of crude oil are processed. The facility differs from the average refinery in that we concentrate on maximizing yield of gasoline from crude oil, due to the specific needs of the Ohio market. We produce ~75% gasoline from crude oil, in contrast to a national average of ~50%.

The Toledo facility is a very modern, efficient refining system. The system includes the first commercial Hydrocracker built in the world (1962). This Hydrocracker was built to convert 7500 bbl/d of diesel fuel/heating oil into gasoline. (SOHIO had found the market for gasoline to be growing at a faster rate than the market for heating oil, putting us out of balance with our customers' needs.) After construction of this facility in 1962, we eventually increased throughput to ~10^4 bbl/d.

SOHIO volunteered to make its facility available (on a "break-even" basis) to the Navy for processing the raw shale oil. It is important to remember that the facility was not built for processing oil, but for processing a much easier-to-handle material. On the other hand, it was probably the best commercial facility available. Of course, one could design and build a facility much better suited for the characteristics of shale oil. Thus, we can assume a difference in the yield and quality of products from this facility vs. products from a commercial unit designed to process shale oil.

SPECIAL CONSIDERATIONS IN REFINING SHALE OIL

Table 4-1 summarizes the characteristics of shale oil vs. typical crude oil. In general, shale oil is much heavier than even the heaviest crude oil. Shale oil contains less of the gasoline and kerosene kinds of materials and more of the heating oil and heavier kinds of materials.

The major problem in refining raw shale oil is the nitrogen content. The 2% nitrogen is some 10 times higher than the nitrogen content of most crude oils (see Table 4-1). In refining, most crude oils undergo catalytic processing, and most currently-used commercial catalysts are subject to poisoning from nitrogen and the by-product ammonia (NH_3). As a result, nitrogen is the "bad actor" in shale oil refining.

The sulfur content of raw shale oil is relatively low in comparison to that of high-sulfur crude oils. Certain metals are substantially higher (in particular, arsenic and iron). These present problems during processing with normal kinds of refining equipment.

STEPS IN THE SOHIO REFINING PROCESS

This subsection describes the processing scheme used to refine the 10^5 bbl. (Actually, 7.8×10^4 bbl were processed.)

Raw shale oil received from Colorado was accumulated in a large tank over a period of 3 to 4 months. The oil was heated to settle out water, but Paraho

TABLE 4-1. CHARACTERISTICS OF SHALE VS. CRUDE OIL

Parameter	Typical Paraho Shale Oil	Typical Crude Oil	
		Heavy, Sour	Light, Sweet
Specific Gravity (°API)	20	25	35
Distillation (°F)			
Initial Boiling Point	375	100	75
10%	520	250	200
50%	790	750	550
90%	1015	1050	1000
Basic Sediments and Water (% by vol.)	0.1	0.2	0.2
Pour Point (°F)	85	10	-10
Sulfur (% by wgt.)	0.7	2.5	0.3
Nitrogen (% by wgt.)	2.1	0.3	0.05
Oxygen (% by wgt.)	1.4	-	-
Ash (% by wgt.)	0.01	-	-
Metals (ppm)			
Arsenic	10	Nil	Nil
Nickel	2	25	5
Iron	38	10	5
Vanadium	1	100	1

had done such a good job that there was no water. Prior to the shale oil run, the hydrotreating facility was shut down for "turnaround," to clean it up and change the catalysts. Thus, the shale oil was processed with clean facilities and new catalysts.

As shown in Figure 4-1, the process consisted of hydrotreating, fractionation of the hydrotreating products, and acid treating for JP-5 and diesel fuel, the primary products being sought. Tankage was included following fractionation, because there was only a single acid treater and it was necessary to block through first one product and then the other. Some of the lighter material (lighter than JP-5) was returned to the refinery pool, to end up in gasoline. The heaviest part was sent to the refinery's heavy fuel pool. The sludge produced in acid treating was sent to a landfill.

We had planned to make small amounts of JP-4 and JP-8, but mechanical difficulties brought us to the end of the run sooner than expected.

Figure 4-2 outlines the hydrotreating process. First, the raw shale oil was pumped through a guard filter. After the guard filter, the filtered raw shale oil was mixed with recycle gas having a high concentration of H_2 (~80% H_2, with the remainder light hydrocarbons), heated to ~700°, and passed through a multi-bed reactor. The multi-bed reactor used a commercially-available hydrotreating catalyst with a high concentration of nickel and molybdenum. (Any number of available catalysts could have been used.) At 700°, with the catalyst, with the H_2, and at a pressure of ~1500 lb/in^2g, reasonably good conversion of sulfur to hydrogen sulfide (H_2S) and nitrogen compounds to NH_3 was accomplished. A still better job could be achieved in a commercial operation using higher pressures, better catalyst, and more catalyst. These improvements would permit higher conversion of nitrogen compounds to NH_3.

An exothermic reaction took place in the reactor. Interbed cooling was employed to limit temperatures (deactivation occurs with excessive temperature). A stream of cool H_2-rich gas cooled down the products after the first bed and after the second bed.

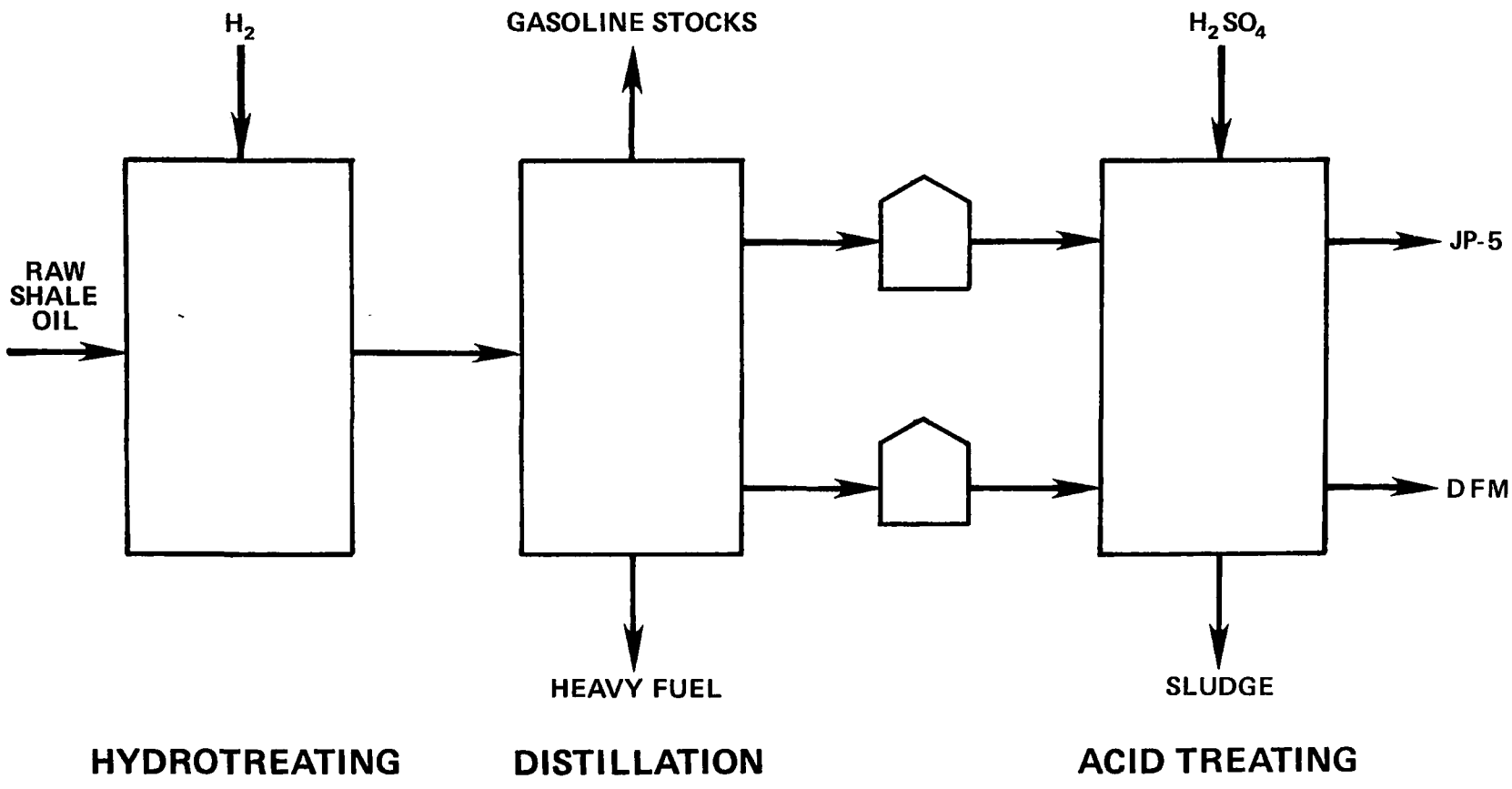

Figure 4-1. Toledo refinery block flow.

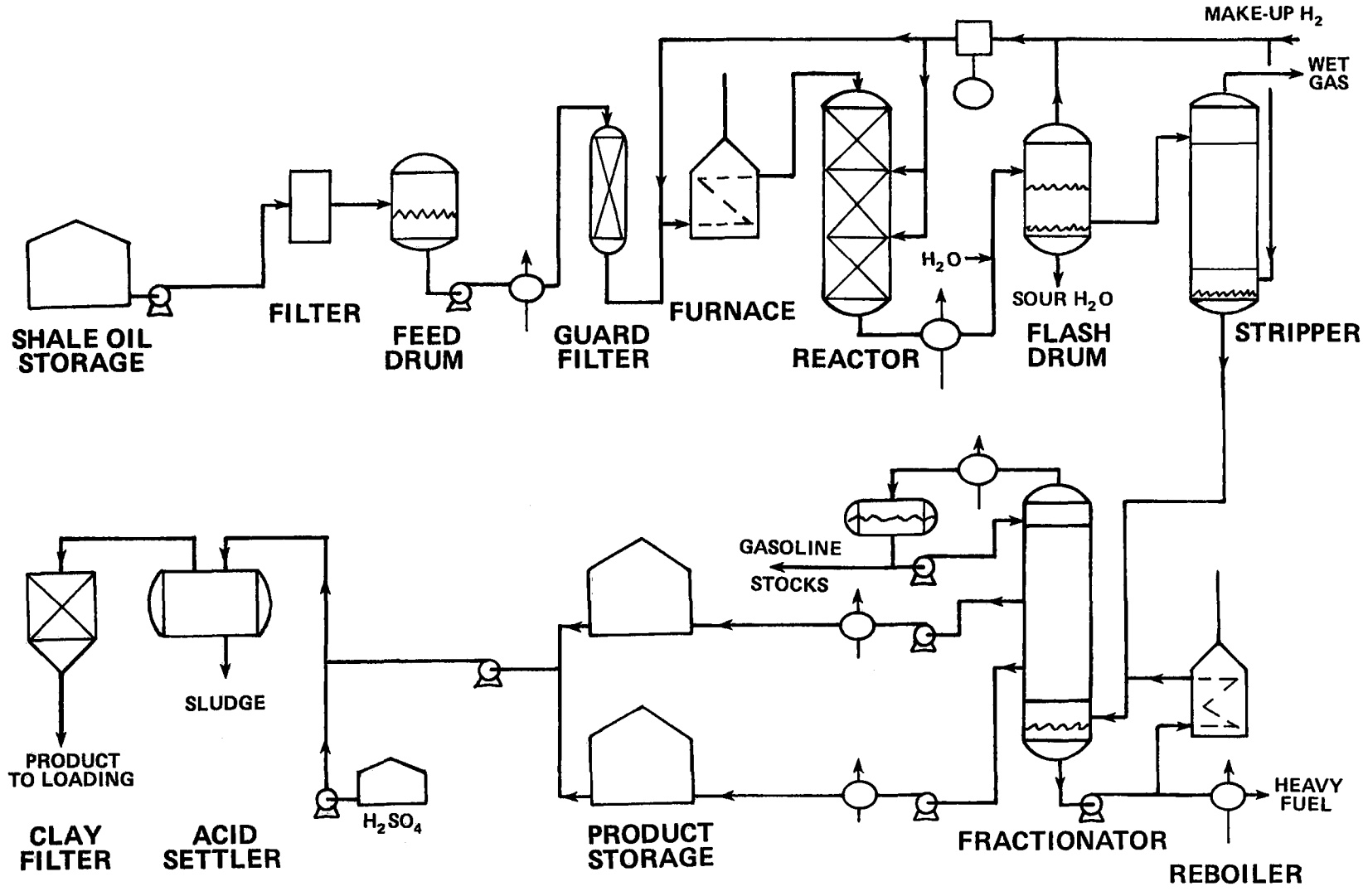

Figure 4-2. Toledo refinery process flow.

After completion of the hydrotreating reaction in the reactor, the next step was to separate the products by adding water, which absorbed NH_3. The water was separated from the oil in a high-pressure separator. The sour water containing NH_3 and some of the H_2S was sent to a stripper to remove these impurities. The excess H_2 in the reactor (over and above the stoichiometric requirements of the reactions) was recirculated, replacing the amount that had been consumed with make-up H_2.

The cool, dewatered oil then flowed to a stripper, where any remaining H_2S and some of the lighter hydrocarbons were removed. The product fractionator was a conventional reboiled and refluxed 50-plate fractionating tower. Four products were produced: a light gasoline stock (lighter than JP-5), the JP-5 product, the diesel fuel product, and the bottom residual material. The tower design did not permit maximization of JP-5 and diesel fuel yields.

From storage tanks, the products were pumped through a simple acid-treating system in which the oil was merely contacted with 93% sulfuric acid (H_2SO_4) to remove residual nitrogen compounds. This acid treating was thorough but not very efficient: 5 to 10% of the product ended up as an acid sludge. Final treating was accomplished in a clay filter using a very fine, natural kind of clay (Attapulgus) to remove any residual trace amounts of acid or sludge.

The final task was to transfer the final products to the Navy by tank car. Preliminary testing indicated all products to be well within Navy specifications.

5. WORK PLAN FOR SHALE OIL STUDY, BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY

T. K. Rao
J. L. Epler

Biology Division
Oak Ridge National Laboratory
Post Office Box Y
Oak Ridge, Tennessee 37830

INTRODUCTION

The principal focus of the Oak Ridge National Laboratory (ORNL) Shale Oil Study is the testing of primary effluents and products for potential effects on man. This portion of the evaluation of Paraho samples concerns questions of relative toxicities of process materials and refinery products.

We propose a parallel, two-level program to expeditiously and cost-effectively answer these questions. Level One is cellular bioassays. These assays will accumulate base-line data on typical effluents and emissions and ascertain how the relative toxicities of major effluents and fractions vary with changes in process conditions. In addition, biological effects studies using cellular assays will provide an essential data base for eventual correlation with acute and chronic toxic effects in whole animals.

Level Two consists of mammalian toxicity bioassays. These assays will involve characterization of the acute, subacute, and chronic toxicities of primary process precursors and products. As data from the analytical chemistry and cellular bioassay programs become available, this information will help in

determining whether additional evaluation of the process by other materials or tests is indicated.

The various assay systems and their application to appropriate test materials or selected active compounds representative of the biohazard present are divided into two categories: (1) testing that is specifically applicable to shale oil, and (2) research or validation that is applicable to the ongoing generic approach of the U.S. Department of Energy (DOE) and other agencies in health effects studies of synthetic fuel technologies. For effective evaluation of the facilities and processes, the two approaches must interrelate and reinforce each another. This paper discusses only the segments necessary to gather specific, comparative data on shale oil samples.

Since even the cellular bioassays are only predictive (and, at that, still developmental in nature), this program is offered only as a practical use of state-of-the-art assays. Considerable basic research must parallel these screening efforts in order to reflect accurately on the question of environmental acceptability of various liquefaction and shale oil processes.

LEVEL ONE: CELLULAR BIOASSAYS (J. L. Epler, Principal Investigator)

Relationship to Health Effects Assessment

These tests are intended to function as (1) predictors of profound long-range health effects such as mutagenesis and/or carcinogenesis, (2) a mechanism to rapidly isolate and identify hazardous biological agents in complex mixtures, and (3) a measure of biological activity correlating base-line data with changes in process conditions. Since complex mixtures can be fractionated and approached in short-term assays, information reflecting on the actual compounds responsible for biological effects may be accumulated. Thus, tests in this category will (4) aid in setting priorities for (a) further validative testing, (b) testing in whole animals, and (c) more definitive chemical analysis and monitoring.

Tables 5-1 and 5-2 list the tests to be applied and materials to be tested.

TABLE 5-1. BIOASSAYS TO BE APPLIED (LEVEL ONE)
AT OAK RIDGE NATIONAL LABORATORY

Screening Bioassay (all samples and fractions)
Salmonella
Yeast Gene Mutation (selected samples and fractions)
DNA Repair
Cytotoxicity
Teratogenesis
Validative Assays (selected fractions)
Drosophila
Mammalian Cell Gene
Mammalian Cell Chromosomal (CHO)
Mammalian Cell Chromosomal (Leukocyte)

TABLE 5-2. SAMPLES TO BE BIOASSAYED (LEVEL ONE)
AT OAK RIDGE NATIONAL LABORATORY

Shale Oil Materials
Crude Shale Oil
Hydrotreated Shale Oil
Gas Feedstock
JP-4 Precursor
JP-5 Precursor
JP-8 Precursor
DFM Precursor
No. 6 Fuel Oil
JP-4
JP-5 (Final)
JP-8
DFM
Acid Sludge
Petroleum "Equivalents"
JP-4
JP-5
JP-8
DFM

Confirmation with a Battery of Tests

Because of the intrinsic limitation of each mutation assay, testing with only one microbial system has often led to faulty conclusions for pure compounds. To overcome this shortcoming, we will employ short-term mutagenesis and/or DNA repair assays to comprehensively screen for both mutagenic and carcinogenic hazards in primary effluents and potential fugitive emissions. Some segments of the battery of assays will be used only on selected active compounds as determined by the coupled effort of chemical and initial biological screens. The actual components will then be characterized as either highly purified fractions or actual pure chemicals. Thus, feedback to chemical screening will become a feasible monitoring method.

Selected samples will also be assayed in cultured mammalian cells. Two major biological end points will be under surveillance: gene mutation and cytogenetic damage. The decision to apply these important assays will be a function of the overall toxicity of the sample; conceivably, only pure isolated and identified components will be tested.

With substantial progress, these ongoing EPA-DOE-cosponsored research activities will benefit from knowledge gained by biological and chemical evaluation of coal liquid test materials. In a reciprocal sense, these studies will feed back into an overall assessment of the hazards of the materials.

Fractionation Methods

Development of a standardized methodology for biopreparation fractionation will be approached through comparison of multiple samples (final shale oil products, crude oils, and precursor products). As shown in Figure 5-1, this method will involve: (1) removal of volatile materials by distillation, (2) collection of distillate, (3) acid/ether extraction, (4) alkaline/ether extraction, and (5) LH-20 chromatography of neutrals via isopropanol and acetone to yield (a) aliphatic, (b) aromatic, and (c) polyaromatic fractions. The array of "oil" samples will be evaluated with multiple extractions and multiple bioassays.

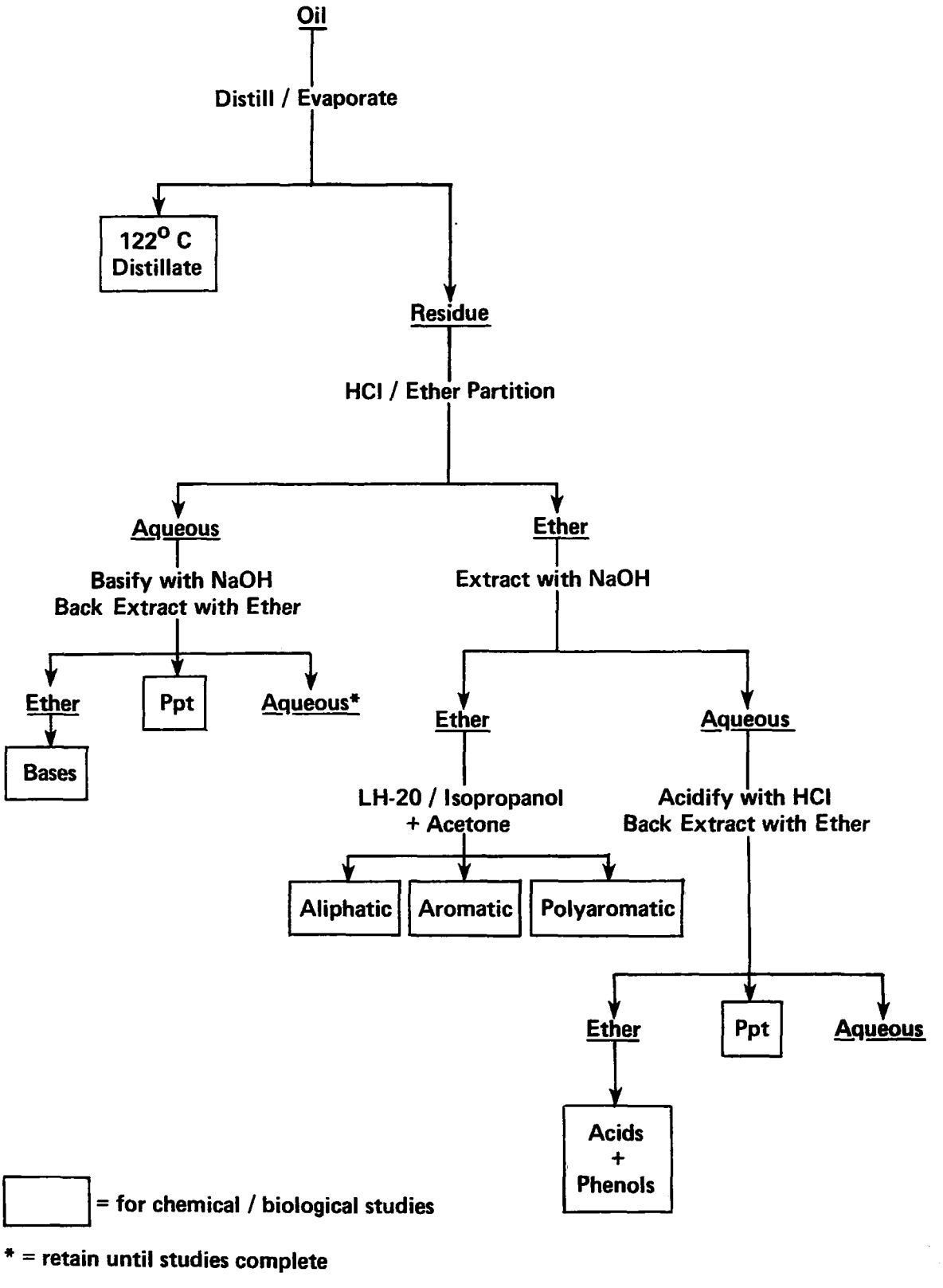

Figure 5-1. Fractionation scheme for shale oil study at Oak Ridge National Laboratory.

In previous studies, ether-soluble base (ESB) fractions of several crudes and aqueous wastes fractionated by a solvent partition method exhibited high biological activities as measured by the Ames microbial mutagenesis test. The ESB fractions of various shale oils will be chosen for subfractionation to isolate and identify the mutagenically active components.

Expected Results

Fractions showing biological activity will be chemically characterized (identification and quantitation when required) to determine the possibly responsible constituents. Further subfractionation will be carried out as necessary, and eventually we will obtain an estimate of relative mutagenicity (potential biohazard). By fractionating and subfractionating active samples, mutagenic activity will be located according to chemical type. (Thus, compounds in most active fractions will require chemical definition.) At this point, we will test the activity of known and newly identified individual compounds in the subfractions and attempt to correlate these results with whole-animal carcinogenicity data. This procedure will provide information on relative hazard in addition to identification of defined biohazards.

LEVEL TWO: MAMMALIAN TOXICITY BIOASSAYS

To be coordinated with the cellular bioassays, the mammalian toxicity bioassays will involve characterization of acute, subacute, and chronic toxicities of a limited number of primary process materials. The samples tested will be materials for which there is a high probability of direct or indirect human exposure. Information from the analytical chemistry, area monitoring, and cellular bioassay programs will guide decisions on whether a thorough evaluation of the process will require additional materials or tests.

The authors recognize the extreme importance of Level Two and also the limited opportunity to obtain representative samples. Accordingly, we expect other EPA/DOE/American Petroleum Institute programs to supplement and consider alternatives to our studies.

Acute Mammalian Toxicity (H. P. Witschi, Principal Investigator)

The following compounds will be tested: (1) retort oil, (2) hydrotreated product, and (3) No. 6 fuel oil. Testing will include: (1) acute oral median lethal dose (LD_{50}) in mice, (2) acute skin toxicity in rats, (3) primary skin and eye irritation in rabbits, and (4) dermal sensitization in guinea pigs.

Acute Oral Toxicity—

Graded doses of the three fractions will be administered by gavage to young male and female mice. The animals will be observed for 2 weeks after dosing or until all signs of reversible toxicity subside, whichever occurs later. The LD_{50} with confidence limits will be calculated.

Acute Dermal Toxicity—

Test agents will be applied to the skin of rats. Except for the different species, the protocol will be essentially the same as in the acute oral toxicity study.

Primary Eye and Skin Irritation—

Test animals will be young albino rabbits. To evaluate eye irritation, each agent will be placed on the everted lower lid of one eye and the lids then gently held together. The contralateral eye will remain untreated as a control. Ocular lesions will be read and graded according to standard procedures during an observation period of up to 14 d.

Primary skin irritation will be evaluated by introducing each test substance onto clipped skin under a 1-in^2 gauze patch. The test substance will be kept in contact with the skin for 24 h. Signs of irritation will be observed and scored until all irritation subsides.

Dermal Sensitization—

Albino guinea pigs will be sensitized 3 times weekly for 3 weeks by intradermal injection or topical patch application. Following the 9th sensitizing treatment, the animals will be set aside for 2 weeks and then challenged by a final injection. Erythema, edema, and other lesions will be scored according to standard procedures at 24 and 48 h after each application.

Acute, Subacute, and Chronic Dermal Toxicity (J. M. Holland, Principal Investigator)

These studies will evaluate the skin penetrability, distribution, and persistence of Paraho shale oil crudes and refined products and establish the correlation between these parameters and specific activities of the whole mixtures, both as skin irritants and as epidermal carcinogens, in vivo.

The following samples will be evaluated: raw crude, hydrotreated crude, No. 6 fuel oil, and DFM.

In Situ Skin Fluorescence—

We have developed a method using native fluorescence to follow the movement of synthetic crude oils through intact skin. The method may also be used to evaluate various barrier creams or cleanup procedures, as well as to quantitate differences in bioavailability between materials.

Using this method, we will apply known amounts (per unit area) of raw crude, hydrotreated crude, and each of the finished products to shaved mouse skin. At 24-h intervals animals will be killed, the skin excised, and frozen sections examined to quantitate levels of fluorescing constituents trapped in situ within sebaceous glands. Once in the sebaceous glands, materials can escape by only two significant pathways: metabolic clearance or mechanical excretion onto the skin surface. Our evidence suggests that, after an initial equilibration period, the sebaceous gland becomes a reservoir for hydrocarbons, and surface concentrations are maintained as a result of the slow (days) but constant secretion of sebaceous lipid containing residual fluorescing hydrocarbons. It is likely that loss from the skin surface is much more dynamic and is mediated through normal desquamative and mechanical processes. One of the things we will learn from the assay is whether retention or trapping of fluorescent materials is greater for some crudes and, if it is, the degree to which this phenomenon correlates with skin irritation or carcinogenesis or both. Some available data suggest a positive correlation between trapping/persistence and toxicity/carcinogenicity, although too few samples have been tested to allow generalization (Holland et al. 1979d).

In Vitro Metabolic Profile—

Our laboratory has developed a simple and efficient method to assess the capacity of native material to modify the metabolism of marker polycyclic aromatic hydrocarbon (PAH) compounds in intact skin. To date, studies have been performed exclusively with labeled benzo(a)pyrene (BaP), but we are extending our observations to other "off the shelf" marker PAH's, each of which reflects a particular metabolic pathway.

Using short-term organ cultures, we will compare the various crude, hydrotreated, and finished products with respect to extent, direction, and nature of influence on overall rate of BaP metabolism. Assuming funds for necessary equipment can be obtained, we plan to compare BaP metabolic profiles obtained in the presence of materials in both mouse and human skin. These assays may provide information on whether the mixtures contain modifiers of PAH metabolism. By comparing rates and profiles for a range of whole crudes as well as the Paraho shale oil and products, we will determine whether significant metabolic disparities occur and what effect, if any, they have on biological potency in vivo.

Pulse Skin Carcinogenesis Bioassay—

Previous experience with prototype whole synthetic crude oils has provided information sufficient for an adequate provisional assessment of relative carcinogenic potency of related materials. This assessment has been achieved within a comparatively short period of time and with reduced test material requirements. We have determined that synthetic crudes differ markedly in capacity to evoke direct skin irritation. In addition, we have obtained evidence suggesting that this in vivo cytotoxicity may inhibit expression of skin neoplasms (Holland et al. 1979d). Because PAH's are also toxic following metabolic activation (Nebert et al. 1977), it follows that tumors are expressed only if neoplastic cells are differentially refractory to continued application of the carcinogen (Farber and Solt 1978) or if carcinogen application is discontinuous. Our approach to bioassay of complex and variably cytotoxic materials strives to maximize the probability of tumor expression while minimizing cytotoxicity and preserving the data's relevance to assessing potential

consequences of occupational cutaneous exposure (which would be episodic rather than continuous).

In our method, groups of animals are exposed either 2 or 3 times weekly to graded doses of the materials diluted in an appropriate solvent. The highest dose is one that can be tolerated without frank erosion or ulceration of the skin. For moderately carcinogenic crudes, >1/3 of the initial population will develop tumors within 32 weeks (Holland et al. 1979b, 1979c, 1979d). By comparison, our C3H mouse has an average tumor latency of 16 weeks at 50 g BaP 3 times/week (Holland et al. 1979a). At 20 or 30 weeks, exposure is discontinued and mice (with and without tumors) are held for an additional 20 weeks to assess the clinical progression of induced neoplasms in the absence of continued exposure. Following this clinical observation phase, surviving mice are killed and those with skin tumors examined for signs of metastasis. All mice (including those that died during the course of the study) are examined for signs of systemic pathology.

Our resources for tests of this nature are extremely limited. Therefore, we will evaluate only the raw crude, hydrotreated crude, No. 6 fuel oil, and DFM. It may be possible to consider the remaining materials in a second cycle of tests. Our reason for selecting No. 6 fuel oil and DFM for carcinogenicity tests is that their higher boiling range might be expected to make them the most carcinogenic of the various products. In other words, if tests of DFM and No. 6 prove negative, we will be surprised if any of the jet fuels are later found to be more active.

INPUT TO HEALTH EFFECTS ASSESSMENT

The two-level program described above is designed to provide specific information on specific process materials. This generic approach, coupled with chemistry, health effects, and environmental studies, will place synthetic fuel materials into context with other materials and processes for which data are available. Direct information on potential mutagenicity, carcinogenicity, and overall toxicity of the process samples will provide

perspective with respect to other technologies. Comparative information and published data on similar materials will permit an ordered estimate of biohazard for each sample. Our team approach will encourage expedient extrapolation of data on known materials.

The relationship of these screening tests to risk assessment in man remains to be demonstrated, and is the focus of considerable ongoing research. By this we do not imply that the concept of screening is at present invalid, but simply that many tests remain developmental to varying degrees. The shale oil research by Paraho/Standard Oil Company of Ohio represents a major opportunity to demonstrate practical applicability of the "screening approach" in toxicity testing.

REFERENCES

Farber, E., and D. Solt. 1978. A new model for the sequential analysis of chemical carcinogenesis in the liver. In: Slaga, T. J., A. Sivak, and R. K. Boutwell (eds.), Carcinogenesis — A Comprehensive Survey. Vol. II. Mechanisms of Tumor Promotion and Cocarcinogenesis, pp. 443-448. New York, Raven Press.

Holland, J. M., D. G. Gosslee, and N. J. Williams. 1979a. Epidermal carcinogenicity of bis(2,3 expoxycyclopentyl)ether, 2,2 bis(p-glycidyloxyphenyl)-propane, and m-phenylenediamine in C3H and C57BL/6 inbred male and female mice. Cancer Res. 39:1718-1725.

Holland, J. M., R. O. Rahn, L. H. Smith, B. R. Clark, S. S. Chang, and T. J. Stephens. 1979b. Dosimetry of coal and shale derived crude liquids as mouse skin carcinogens. J. Occ. Med. 21:614-618.

Holland, J. M., M. S. Whitaker, and L. C. Gipson. 1979c. Chemical and biological factors influencing the skin carcinogenicity of fossil liquids. Proceedings of Park City Environmental Health Conference, Park City, Utah, April 4-7.

Holland, J. M., M. S. Whitaker, and J. W. Wesley. 1979d. Correlation of fluorescence intensity and carcinogenic potency of synthetic and natural petroleums in mouse skin. Am. Ind. Hyg. Assoc. 40:496-503.

Nebert, D. W., R. C. Levitt, N. M. Jensen, G. H. Lambert, and J. S. Felton. 1977. Birth defects and aplastic anemia: Differences in polycyclic hydrocarbon toxicity associated with the Ah locus. Arch. Toxicol. 39:109-132.

6. SHALE OIL BIOASSAYS AT BATTELLE PACIFIC NORTHWEST LABORATORIES

R. A. Pelroy

Biology Department
Battelle Pacific Northwest Laboratories
Post Office Box 999
Richland, Washington 99352

INTRODUCTION

Efforts by the Biology Department of Battelle Pacific Northwest Laboratories will include *in vitro* mutagenicity, DNA damage, and cellular toxicity testing of Paraho shale oil, intermediate process streams, and refined shale oil products. Chemical fractionation of the complex mixtures will be accomplished by solvent extraction and thin layer chomatography (TLC). The *in vitro* assays coupled to analysis by gas chromatography/mass spectrometry (GC/MS) of crude material, solvent extracts, and TLC fractions will be the main analytical tools for relating activity to chemical species.

SAMPLES TO BE ASSAYED

Our testing regime will examine the unrefined shale oil (starting material), intermediate process streams and by-products, and refined products. Table 6-1 presents an itemized list of these materials. At present, our Department lacks the resources to investigate airborne matter, raw shale, spent shale, and similar materials, although we remain interested in such potential studies.

TABLE 6-1. SAMPLES TO BE BIOASSAYED AT BATTELLE PACIFIC NORTHWEST LABORATORIES

Crude Shale Oil
Hydrotreated Shale Oil
No. 6 Fuel Oil
Gasoline Stock
JP-4 Precursor
JP-5 Precursor
JP-8 Precursor
DFM Precursor
JP-4
JP-5
JP-8
DFM
Acid Sludge
Water (retort oil separation)
Water (from stripper)

FRACTIONATION METHODS

Our Department currently employs two fractionation schemes to break down complex hydrocarbon mixtures into component chemical classes. The scheme currently used for most of our work is a simple acid-base solvent extraction procedure that yields acidic, basic, neutral, and polynuclear-aromatic-hydrocarbon-containing fractions. These fractions are not subdivided (e.g., into weak acids, weak bases, etc.) and are thus somewhat less refined than samples obtained with a "Swain-type" procedure. Our main purification step occurs with TLC separation of the components in the fractions.

A second fractionation method is currently being applied to shale oil and may eventually replace the simple solvent extraction procedure, provided we can show significant improvements in yields or resolution of biologically active materials. This scheme is based on Swain-type solvent extraction of complex mixtures followed by column fractionations similar to those developed

at Oak Ridge National Laboratory. We plan to carry out direct comparisons of the two types of fractionation schemes (i.e., simple solvent extraction versus solvent extraction-column fractionation) in terms of the materials yielded for bioassay.

BIOASSAYS TO BE APPLIED

Table 6-2 lists the bioassays that our Department will use to assess the various samples. The main test system will be the Ames Salmonella assay; direct comparisons will be made with the other systems listed.

TABLE 6-2. BIOASSAYS TO BE APPLIED AT BATTELLE PACIFIC NORTHWEST LABORATORIES

Test	System
Bacteria for mutagenesis (Ames assay)	Salmonella
Mammalian cell cultures for mutagenicity at hgprt	CHO cells
Mammalian cell cultures for SCE	CHO cells
Mammalian cell cultures for toxicity	CHO cells

SUMMARY

By way of summary, Figure 6-1 presents a schematic diagram of our intended protocol for assessing shale oil materials. To reiterate, we will emphasize a simplified solvent extraction procedure with heavy reliance on TLC in combination with GC/MS. Our approach will be essentially analytical, focusing on the structural relationships that may relate mutagenic activity to chemical species. The Ames assay will serve as the main connecting link between the chemistry and the biology.

SAMPLES	ASSAYS[a]	FRACTIONATION[b]
Unrefined shale oil, intermediate process streams and by-products, refined products	Ames, CHO (mutation, SCE, toxicity)	Solvent extraction to yield acid, base, neutral (etc.) fractions; TLC to yield subfractions; GC/MS analysis of subfractions

[a] Heaviest reliance will be placed on the Ames assay, with direct comparisons to other assays (e.g., Ames back mutation vs. CHO SCE assay, etc.).

[b] GC/MS analysis will be routinely used only on samples yielding positive *in vitro* assays.

Figure 6-1. Schematic representation of procedure for assessing shale oil materials at Battelle Pacific Northwest Laboratories.

7. APPLICATION OF A BATTERY OF SHORT-TERM BIOASSAYS FOR TESTING THE GENETIC TOXICITY OF PARAHO SHALE OIL PRODUCTS

F. T. Hatch
H. Timourian

Lawrence Livermore Laboratory
University of California
Biomedical Sciences Division
Post Office Box 5507
Livermore, California 94550

INTRODUCTION

The objective of this project is to determine the relative mutagenicities and genetic toxicities of crude, hydrotreated, and refined shale oil products from the Paraho surface retort and to compare the potential health hazards of these materials with hazards of similar petroleum-derived materials. Application of a battery of bioassays consisting of a standard microbial test and *in vitro* and *in vivo* mammalian systems will provide a basis for estimating human health hazards.

A substantial portion of the Biomedical Sciences Division program at Lawrence Livermore Laboratory (LLL) is devoted to integrated application of cell biology, analytical cytology, and biochemical techniques to problems of environmental mutagenesis, carcinogenesis, and injury to the reproductive system. Most of the test systems in the available battery of bioassays (Table 7-1) were developed and validated at LLL. This battery emphasizes mammalian systems and includes both *in vitro* (cell culture supplemented with metabolic activation) and short-term *in vivo* components.

TABLE 7-1. BIOASSAYS AVAILABLE AT LAWRENCE LIVERMORE LABORATORY

Test	System	End Point or Parameter Measured	Average Process Time (weeks)	Cost Range[a] (dollars/sample)	Testing Capability (samples/ yr/FTE)
Bacteria for mutagenesis (Ames assay)	*Salmonella* strains that require histidine	Growth of reverse-mutant colonies in the absence of histidine	1	350-600	150-200
Cultures (mammalian cells) for:					
Toxicity	CHO and mouse hepatoma cells	Growth of cell colonies in presence of test substance	2	350-600	150-200
Mutagenicity	CHO cells	Growth of drug-resistant mutants in presence of lethal dose of drugs	8	2500-5000	20-30
Chromosome damage	CHO cells	Sister chromatid exchange in cells	2	700-1500	100
Whole animals for:					
Chromosome damage	Mice	Sister chromatid exchange in bone marrow cells	2	800-1500	90
Sperm morphology	Adult male mice	Abnormal morphology of epididymal sperm	8	3000-6000	10-15
Oocyte depletion	Newly born female mice	Survival of primary oocytes	4	1500-3000	25-30

[a]Cost includes testing at several doses to give a dose-response curve.

Five types of bioassays are available for the project:

(1) Microbial mutagenesis by detecting revertants to histidine independence in Salmonella typhimurium (Ames assay)

(2) Mammalian cellular toxicity by measuring differential toxicity in cultures of mouse hepatoma cells lacking or containing aryl hydrocarbon hydroxylase and cultures of Chinese hamster ovary (CHO) cells with and without defects in repair of deoxyribonucleic acid (DNA)

(3) Mammalian cellular mutagenesis by measuring the frequency of mutations at multiple gene loci in CHO cells (hprt, aprt, ATPase, tk)

(4) Mammalian cellular and genetic toxicity to germ cells by measuring the frequency of induction of abnormal head shape in sperm and killing of primary oocytes in vivo

(5) Chromosomal injury and misrepair by measuring the frequency of induction of sister chromatid exchange (SCE) in vivo

AVAILABLE BIOASSAYS

Ames Assay

This test determines mutation induction (reversion) in histidine auxotrophs (histidine requiring strains) of Salmonella typhimurium. The bacteria are exposed to the shale oil materials; revertants that survive and form colonies in histidine-free media are counted. The number of revertant colonies represents a direct measure of induced mutation. Since some mutagens require enzymatic activation, bacteria are exposed with and without S9, a preparation of rat liver microsomes (Ames et al. 1975).

Mouse Hepatoma Cell Assay

Mouse hepatoma cells permit rapid detection of benzo(a)pyrene (BaP) and other polycyclic aromatic hydrocarbons in complex mixtures. Two genetic cell lines, one sensitive and the other resistant to BaP (Hankinson 1979), are exposed to the shale oil materials. A differential toxicity response provides a rapid screening method (Figure 7-1). Since the major genetic difference between the two strains is the ability to activate BaP, this test does not require addition of a microsomal activating system.

Chinese Hamster Ovary Cell Toxicity Assays

CHO cells can be used to study cytotoxicity in two ways. In the first method, a plating efficiency curve is run at a series of exposure doses to determine D_{37}(M) (the molar dose of agent that kills 63% of the initial cell population). A review of the literature on mutagenesis in mammalian cell cultures (Carver et al. 1979b) and extensive data of June Carver (LLL) indicate a high correlation between induced mutation frequency (per mole per liter exposure dose) and D_{37}(M) that is applicable for several genetic markers and several mammalian species (Figure 7-2). Thus, in screening and setting priorities for further testing, this simple and rapid measurement of cytotoxicity is often predictive of mutagenicity, and any errors will be conservative.

The second method employs one or more mutant strains of CHO cells now being developed by Larry Thompson (LLL). These mutants are substantially more sensitive to various classes of mutagens than the "wild"-type strain, presumably owing to defects in DNA repair mechanisms. Thus, differential cytotoxicity between "wild" and mutant cells indicates damage to DNA.

Chinese Hamster Ovary Cell Mutagenesis Assays

CHO cells are incubated with the test material alone or with a microsomal activating system. After exposure, the CHO cells are tested for reproductive ability (by determining plating efficiency) and for specific single step

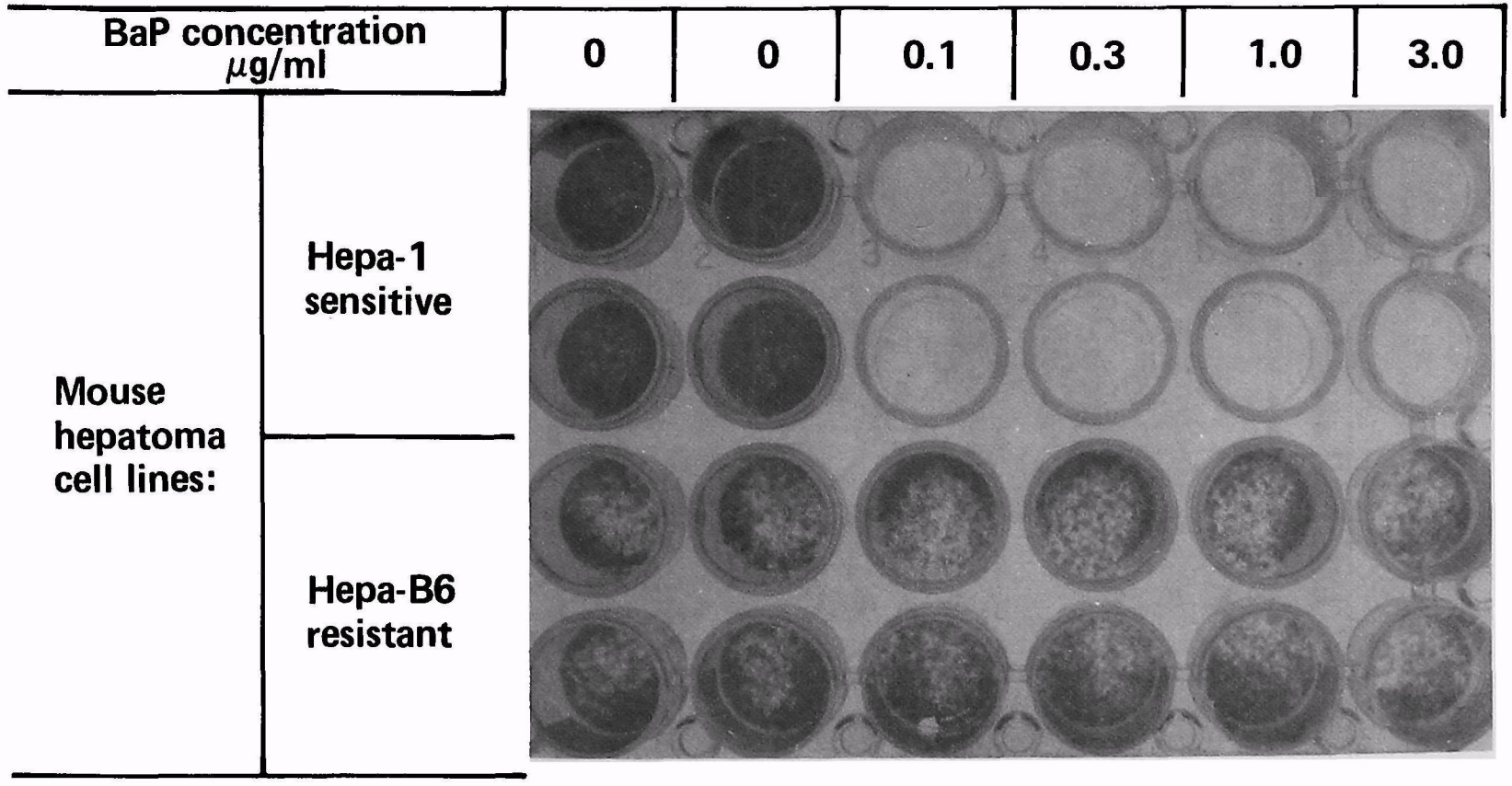

Figure 7-1. Mammalian cellular toxicity assay employing mouse hepatoma cells. Duplicate cultures containing 2 x 10^4 cells/well of either sensitive or resistant cells were exposed to BaP. After 5 d, sensitive cells were killed and resistant cells grew to confluence at all BaP concentrations.

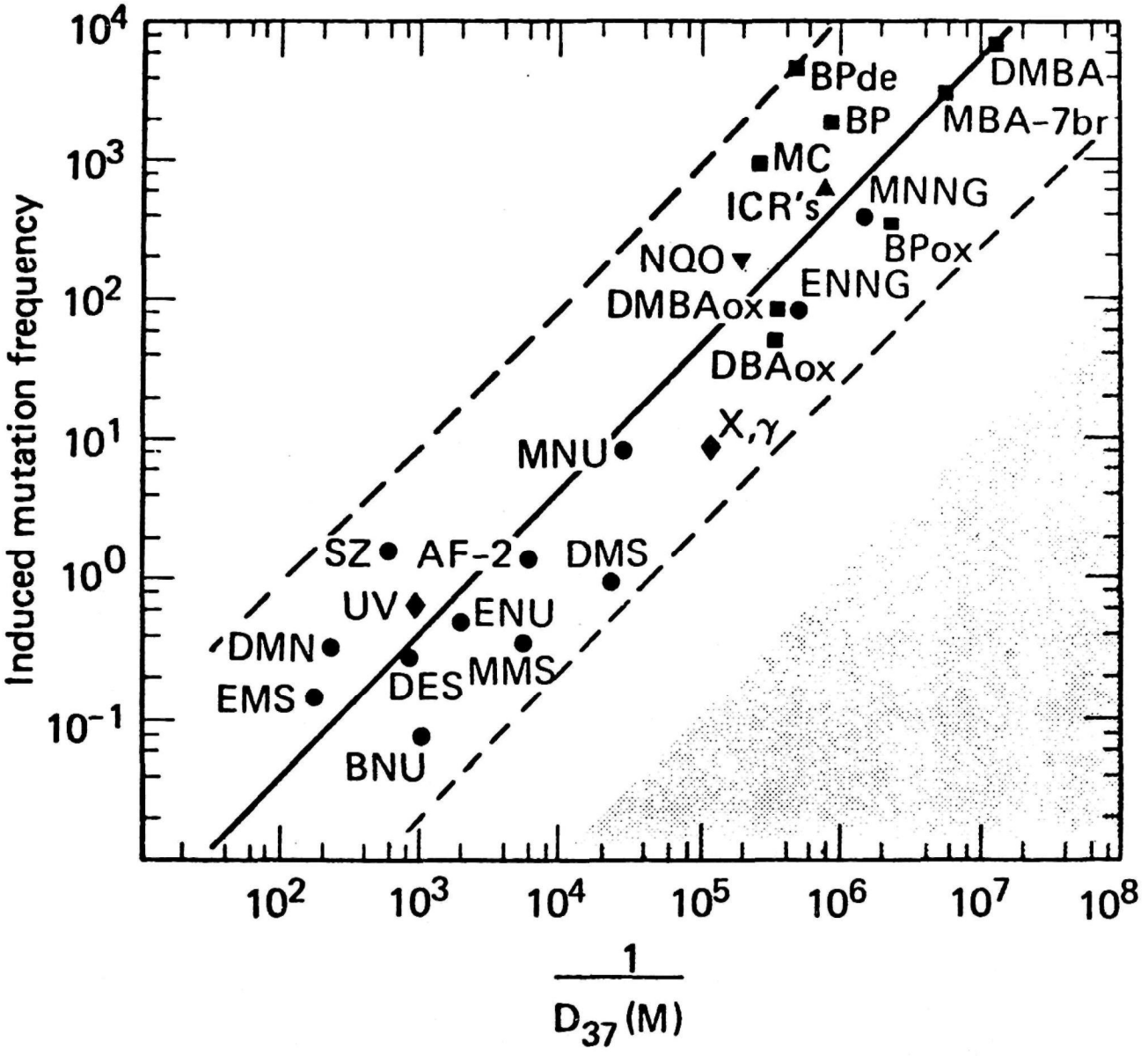

Figure 7-2. Results of simple CHO cell toxicity assay of 22 chemical mutagens. Cytotoxic potency correlates with mutagenic potency as assayed at hprt, aprt, and tk loci in five rodent and human in vitro cell systems. Data for induced mutations are plotted as a function of the reciprocal of $D_{37}(M)$. See Carver et al. (1979b) for details.

mutations at four different loci (by determining resistance to lethal drugs). Plating efficiency provides a measure of toxicity and, in some cases, an indirect measure of mutagenicity. To determine mutagenicity, cells exposed to the test material are subsequently cultured in lethal concentrations of a drug (e.g., azaadenine, azaguanine, fluorodeoxyuridine, or ouabain). Survival and growth in the presence of the drug are a measure of induction of mutation by the test material (Figure 7-3). In general, the effects at different markers are correlated, but recent data (Carver et al. 1979a; Thompson 1979) indicate that certain mutagen classes may show differential effects at the markers, so that the multiple marker CHO assay may provide a broader spectrum to detect mutagens than the commonly used strains. The CHO cell bioassays are also sensitive to mutation induction by metal ions and their methylation products (Taylor et al. 1979a, 1979b).

Mammalian Germ Cell Toxicity Assays

Sperm Head Abnormalities—

Four weeks after *in vivo* exposure to shale oil materials, sperm head abnormalities may be detected in adult male mice (Figure 7-4). The fractions of epididymal sperm that are morphologically abnormal are counted by microscopic observation. Isogenic strains of mice are used because the percentage of abnormal sperm in each strain is constant. The induction of abnormal sperm morphology by mutagens is well documented (Wyrobek and Bruce 1978). Recent studies in industrial populations indicate sensitivity of human spermatogenesis to pesticides and other chemicals (Wyrobek and Gledhill 1978).

Oocyte Depletion—

Following *in vivo* exposure to shale oil materials, oocyte depletion may be detected in newly born female mice (Figure 7-5). Exposure is either direct to the newborn by gavage or indirect (*in utero*) by treatment of the pregnant mother. Mice are sacrificed 14 d after exposure, ovaries are sectioned, and numbers of oocytes are counted. The number of oocytes in the ovaries of an organism is set before birth and normally diminishes at a predictable rate during lifetime. Oocytes are very sensitive to mutagens, and an accelerated rate of depletion may indicate genetic damage (Dobson et al. 1978). The median

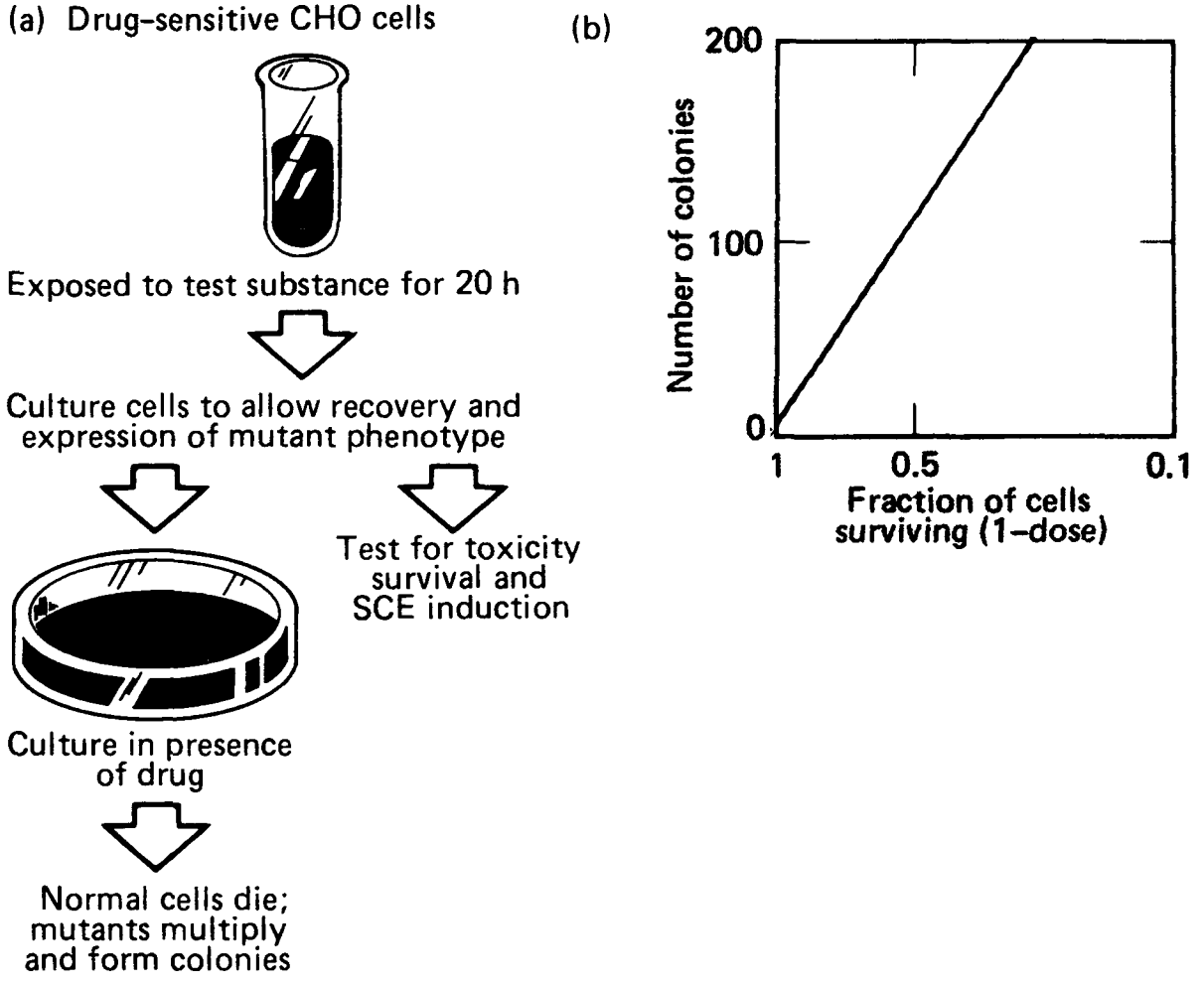

Figure 7-3. CHO cell mutagenesis assay. CHO cells exposed to energy effluents or their fractions are tested for toxicity survival, mutation induction, and SCE. (a) Mutations are detected by cell survival in the presence of lethal doses of drugs such as azaadenine or azaguanine. These drugs are structurally similar to adenine or guanine, bases that make up DNA and RNA (nucleic acids that carry genetic information). A mutation in genes that specify the structure of enzymes using guanine or adenine will prevent the incorporation of the drug into DNA and RNA and make the cells drug-resistant. (b) The number of azaguanine-resistant mutant cells as a function of the mutagen ethyl methane sulfonate (EMS) is shown by plotting the number of mutant colonies vs. the fraction of cells that survived the toxic effects of EMS (survival is inversely proportional to applied mutagen dose).

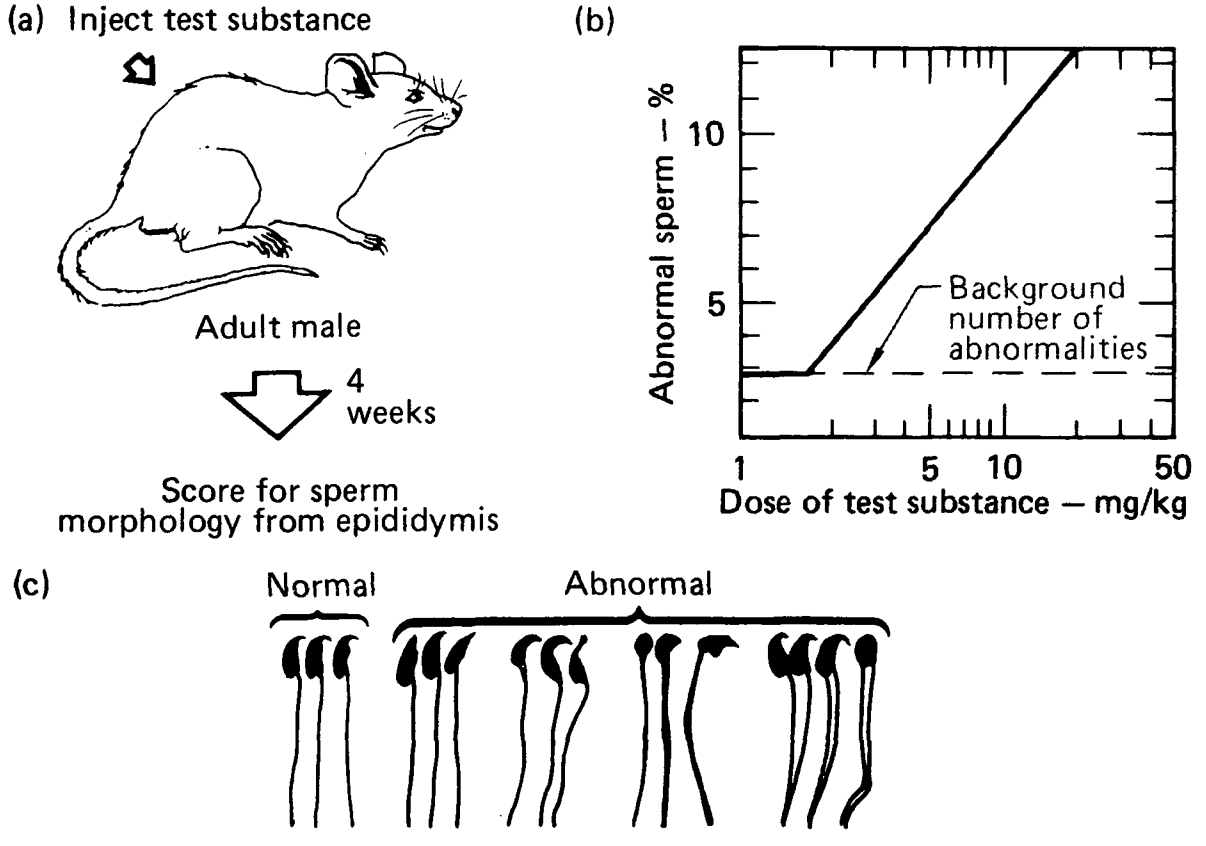

Figure 7-4. Assay for morphological abnormalities in sperm of adult male mice. Induction of abnormal sperm morphology by test substances provides an indication of genetic damage. (a) Sperm development takes 4 weeks; detrimental effects of the test substances are easily detected by counting abnormal sperm after this period. (b) Plot of the effect of 3-methyl cholanthrene on the frequency of abnormal sperm as a function of dose. (c) Typical samples of sperm morphology as seen under the microscope.

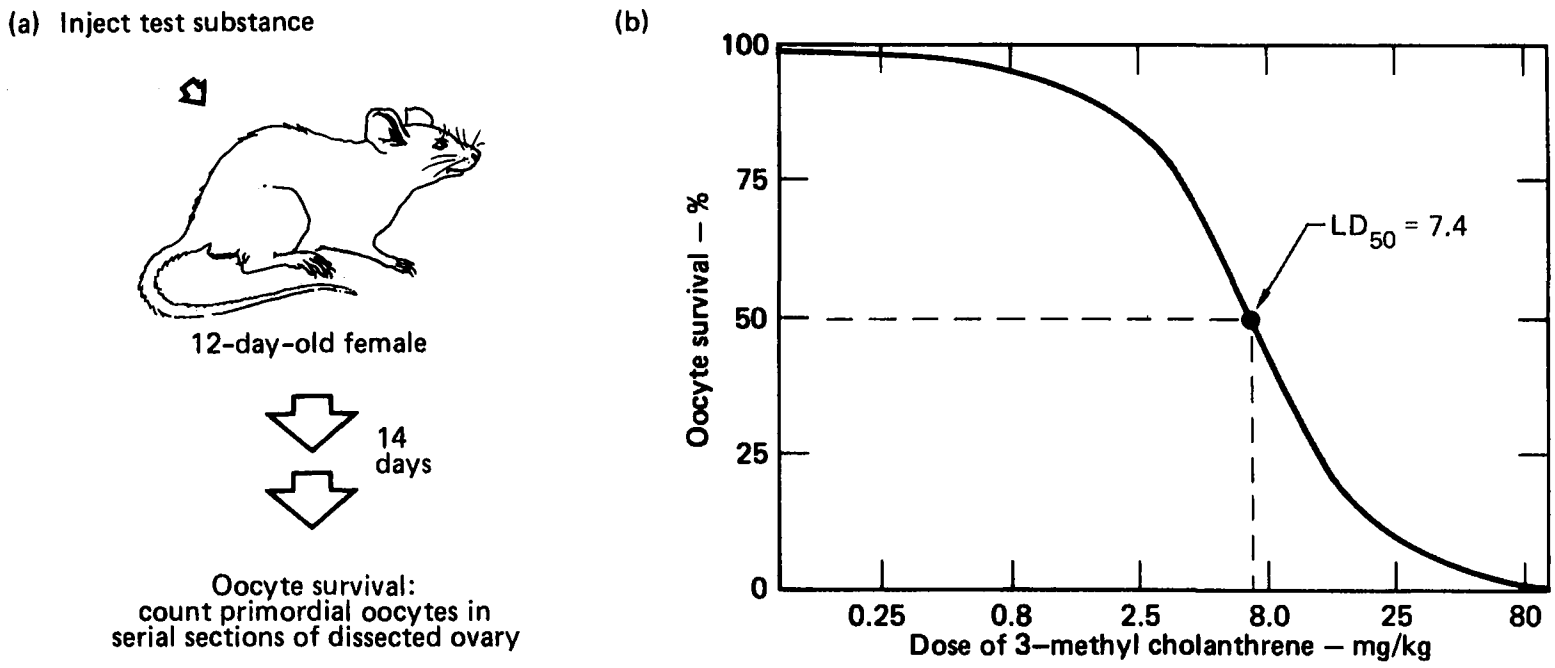

Figure 7-5. Assay for depletion of oocytes in newly born female mice. The highly sensitive oocytes of young female animals are killed by most mutagens. (a) Surviving oocytes are counted in serial sections of the ovary to calculate the LD_{50}. (b) Oocyte survival after injection of different doses of 3-methyl cholanthrene.

lethal dose (LD_{50}) for X-rays is ~5 rad; LD_{50} values for several polycyclic aromatic hydrocarbons range from 1 to 20 mg/kg (representing, in the newborn mouse, a total dose of a few µg).

Sister Chromatid Exchange Assay

SCE's may be assayed in mice after *in vivo* exposure to shale oil materials. At 5 to 8 h before exposure to test material, mice are prepared by implantation of BrdUrd pellets under the skin. Animals are sacrificed 12 to 15 h later and cells in bone marrow examined and scored for SCE's (Figure 7-6). The SCE's (exchanges of segments between sister chromatids) are visible in metaphase chromosomes because BrdUrd incorporated during DNA synthesis stains the new and old chromatids differently. SCE's indicate that genetic damage and repair may have taken place. The number of SCE's has been correlated with the number of mutations in mammalian cell cultures for several mutagens (Carrano et al. 1978). We assume that the relationship that applies in cell cultures will apply in whole animals (Stetka et al. 1978).

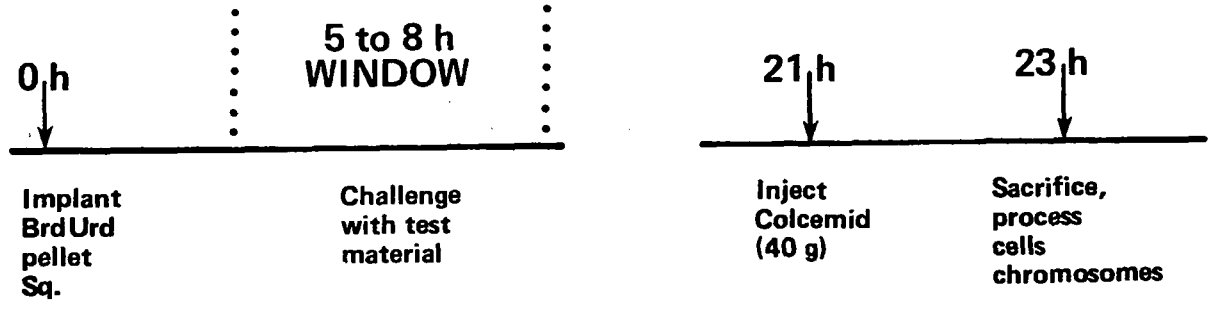

Figure 7-6. Sister chromatid exchange assay. Induction of SCE's is determined after *in vivo* exposures in mice prepared with BrdUrd and later injected with colcemid to arrest systemic cell division at mitosis.

STRATEGY OF APPLICATION

The major biological concern with environmental pollution is widely believed to be DNA damage resulting from low-dose, often chronic exposure. Such DNA damage can cause defects in the information content of the genome. The principal consequences are carcinogenesis in the current generation and an increase in the load of detrimental mutations in future generations.

Since direct measurement of either of these consequences from the large variety of agents of concern is too time-consuming and expensive, a variety of short-term tests was developed to indicate hazard and to aid in setting priorities for definitive testing. An early concept was the "tier" approach, in which application of a simple and rapid test was followed, if positive, by successively more elaborate tests. As time passed, virtually every short-term test proved fallible or even blind to certain classes of agents.

The currently favored strategy is a "sequential battery" in which two or more tests (preferably based upon different genetic principles) are applied at several levels of complexity. Even this approach has drawbacks. For example, Purchase et al. (1976) calculated that application of eight tests in which each has 90% accuracy results in a high probability for identification of all toxic agents. However, 43% of the truly negative agents would also give a positive result in one or more tests of the battery; therefore, careful judgment is required to assess the correct status of some agents. Also, in the sequential battery approach it is probably wise to carry some random samples of initially negative agents forward into certain of the more elaborate assays.

An important consideration in comparing bioassays is sensitivity (i.e., the minimum concentration of an agent that will give a positive response). In this respect, the principal experience of the authors' laboratory at LLL has been with groundwater from wells surrounding the burn zone of an in situ coal gasification experiment at Hoe Creek, Wyoming. Figure 7-7 shows the sensitivities of our assay battery expressed as the reciprocal of parts per million organic matter in sample required for a positive effect. The sensitivities

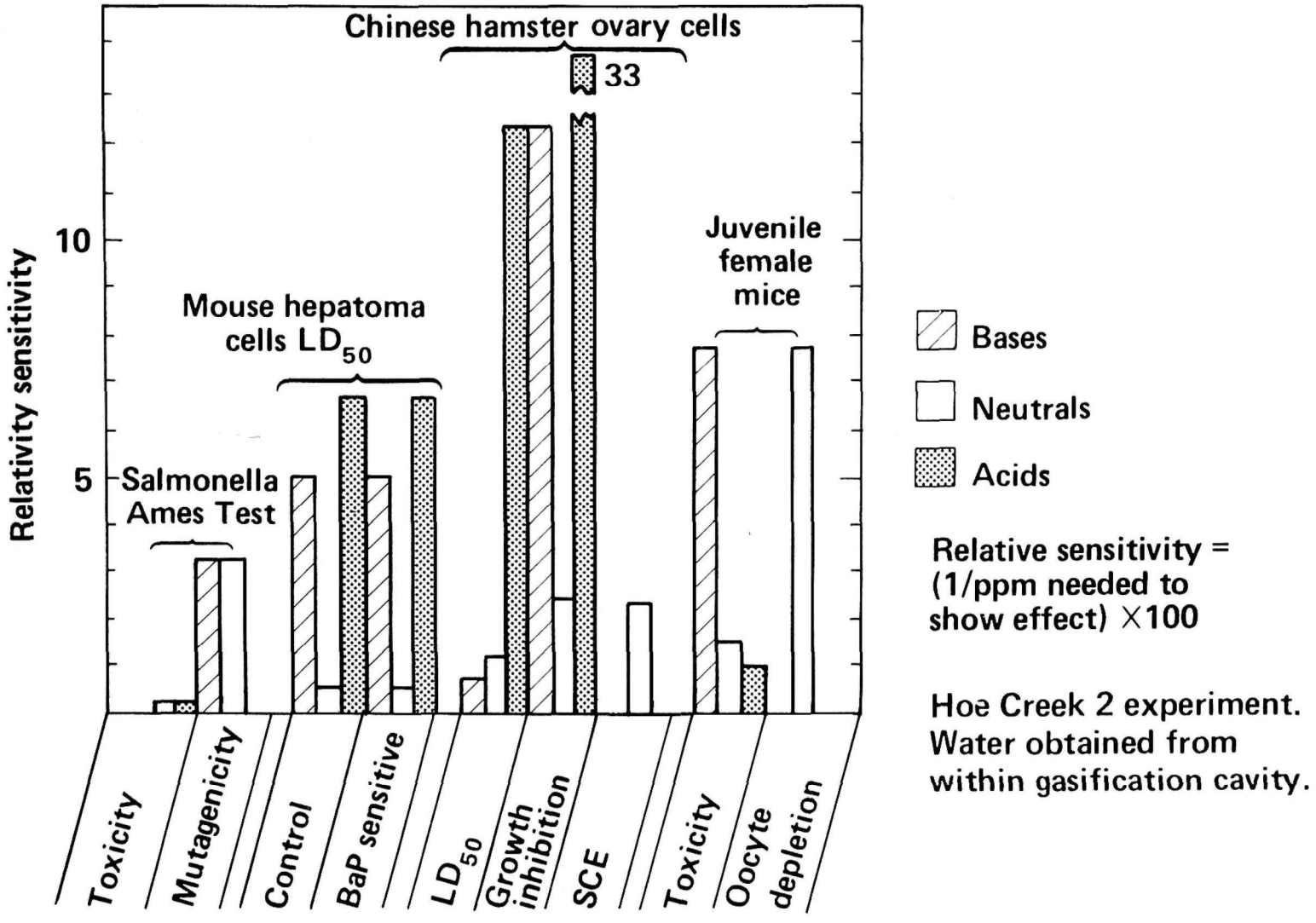

Figure 7-7. Relative sensitivity of different bioassays to chemical fractions from underground water after coal gasification.

of the assays appear roughly similar; however, there is some indication that the two assays for CHO cell toxicity are somewhat more sensitive than the others (including the Ames assay).

The manpower and time requirements for bioassays are, of course, important considerations. In general, except for the Ames assay and the simpler mammalian tests, the assays require ~0.5 man-month for a complete dose response. Turnaround times range from 1 week (for the simpler tests) to ~2 months (for the more elaborate ones).

When the results of a battery of tests are available, there remain the important tasks of assessing human risk from realistic estimates of exposure and of comparing relative risks in alternative courses of action. This new area of genetic toxicology does not yet have a satisfactory theoretical, or even empirical, framework. The authors' Division at LLL has limited experience in the area but expects to become fully involved in the future. In particular, there are strong possibilities for comparison of data from the LLL bioassay battery with data becoming available from short-term genetic tests applied directly in humans exposed to industrial agents or medical therapy. The authors' Division is active in development and validation of some of these tests, and application will greatly facilitate the development of risk-estimation techniques (Carrano 1979; Wyrobek and Gledhill 1978).

SAMPLES TO BE ASSAYED

Initial plans are to assay crude and hydrotreated shale oil samples. Additional samples will be tested if preliminary results from other laboratories (in particular, the laboratory of J. Epler at ORNL) indicate some of the refined shale oil products to also be mutagenic. Initial results from mutagenicity tests will be confirmed in mammalian cell lines and whole-animal tests. Table 7-2 shows a priority matrix of available assays and materials to be tested.

Funding for application of our bioassay battery to samples of fossil fuels or effluents remains quite limited. Furthermore, the number of technology sources competing for our limited manpower and resources is growing rapidly (e.g.,

TABLE 7-2. PRIORITY MATRIX FOR BIOASSAYS AT LAWRENCE LIVERMORE LABORATORY

Bioassay	Sample					
	Crude	Hydrotreated	DFM (pre-acid-treatment)	DFM (final)	Various Jet Fuels	Equivalent Petroleum Products
Ames Assay	A/O	A/O	A/O	A/O	A/O	A/O
Mouse Hepatoma Cell Line	A	A	B	B	B	B
CHO Cell Mutagenicity and Toxicity	A	A	B	B	B	B
SCE in Mice	A	A	B	B	B	B
Sperm Abnormalities	A	A	B	B	B	B
Oocyte Depletion	A	A	B	B	B	B

A = Highest priority.
B = High priority only if positive results are found in other bioassays.
O = May be tested in other laboratories (e.g., J. Epler at ORNL).

Paraho shale fuels, Oxy *in situ* fuels and effluents, LLL retort samples, RCRA leachates, and Rio Blanco *in situ* experiments). To reiterate, the authors consider the highest current priority to be the issue of hydrotreating shale oil to significantly lower its toxicity. Accordingly, our Division will assay selected Paraho samples as appropriate. Particularly for the Navy's concerns, however, the authors regard the study of combustion effluents from these fuels as more important than assaying the neat fuels.

EXPECTED RESULTS

Initially, the Ames assay will be used to determine if hydrotreatment, a necessary step to improve flow and refining characteristics, reduces the mutagenicity of the crude shale oil. Use of the Ames assay on other refined products (as performed by LLL or ORNL) will determine relative mutagenicities.

In vitro bioassays using mouse hepatoma and CHO cell lines will confirm Ames assay results. Previously, matched hepatoma lines (BaP-sensitive and -resistant) treated with graded doses of crude and hydrotreated Paraho shale oil in a continuous 5-d exposure responded similarly in dose toxicity. However, both cell lines were killed at lower concentrations of the crude sample. For example, growth inhibition at 11 µg/ml with the crude sample was comparable to inhibition at 94 µg/ml with the hydrotreated sample. These results indicate that the measured toxicity is not due to BaP-like compounds, but perhaps to substances causing nonspecific toxicity.

In vivo animal bioassays will test the effects on mutagenicity of different routes of administration, access of agents to germ cells, genetic capacity for activation, and various dose rates. In a previous study, whole-animal LD_{50} levels in mice were determined as a preliminary to *in vivo* tests. Juvenile mice were injected intraperiotoneally with single doses of either crude or hydrotreated Paraho shale oil. After 2 weeks, LD_{50} values of 3.8 g/kg body weight (Paraho crude) and 31.7 g/kg body weight (hydrotreated product) were determined. The higher toxicity of the crude was emphasized by the fact that at 13.2 g/kg all animals exposed to crude were dead within 1 week.

In contrast, all animals exposed to the same dose of hydrotreated product were still alive 2 weeks later.

Although the significance of the relative toxicities of crude and hydrotreated shale oil is not yet clear, note that crude was 8 times more toxic than hydrotreated in both systems tested (in vitro hepatoma cell lines and in vivo juvenile mice). This eightfold difference in toxicity is likewise reflected in the relative carcinogenic activity of the two materials. For example, in skin painting experiments using crude and hydrotreated Paraho shale oil, Coomes (1979) reported tumor development in 13% of animals painted with hydrotreated shale oil and 97% of those painted with crude. Such results contribute to an understanding of the spectrum of toxicity of shale oil products in bacterial and mammalian systems, and help to predict potential effects on humans.

In toxicologic studies of synthetic fuels (particularly the work of J. Epler at ORNL and R. Pelroy at Battelle Pacific Northwest Laboratories), the basic alkaline pH fraction appears to have the highest potency; this fraction contains a variety of nitrogeneous heterocyclic aromatic compounds. In a separate project, the authors recently found that the major mutagenic activity produced in cooking beef is also in the basic fraction. Although the genetic toxicology of naphthylamines, azo dyes, and aminobiphenyls has been studied, many classes of organic bases deserve further investigation.

ACKNOWLEDGMENT AND DISCLAIMER

This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore Laboratory under Contract W-7405-ENG-48.

any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately-owned rights."

REFERENCES

Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364.

Carrano, A. V. 1979. Sister chromatid exchange: Relation to mutation and application to human population studies. Abstract, DHEW Subcommittee on Environmental Mutagenesis, May 7. National Institutes of Health, Bethesda, Maryland.

Carrano, A. V., L. H. Thompson, P. A. Lindl, and J. L. Minkler. 1978. Sister chromatid exchange as an indicator of mutagenesis. Nature 271:551-553.

Carver, J. H., G. M. Adair, and D. L. Wandres. 1979a. Mutagenicity testing in mammalian cells. The development and validation of multiple drug-resistance markers having practical application for screening potential mutagens. Preprint UCRL-81703. Lawrence Livermore Laboratory, Livermore, California.

Carver, J. H., F. T. Hatch, and E. W. Branscomb. 1979b. Estimating maximum limits to mutagenic potency from cytotoxic potency. Nature 279:154-156.

Coomes, R. M. 1979. Carcinogenic testing of oil shale materials. 12th Oil Shale Symposium, Colorado School of Mines, Denver, Colorado, April 18-20.

Dobson, R. L., C. G. Koehler, J. S. Felton, T. C. Kwan, B. J. Wuebbles, and D. C. L. Jones. 1978. Vulnerability of female germ cells in developing mice and monkeys to tritium, gamma rays, and polycyclic aromatic hydrocarbons. Develop. Toxicol. of Energy-Related Pollut. CONF-77107, DOE Symp. Series 47, pp. 1-14.

Hankinson, O. 1979. Single-step selection of clones of a mouse hepatoma cell line deficient in aryl hydrocarbon hydroxylase. Proc. Natl. Acad. Sci. 76:373-376.

Purchase, I. F. H., E. Longstaff, J. Ashby, J. A. Styles, D. Anderson, P. A. Lefevre, and F. R. Westwood. 1976. Evaluation of six short term tests for detecting organic chemical carcinogens and recommendations for their use. Nature 264:624-627.

Stetka, D. G., J. L. Minkler, and A. V. Carrano. 1978. Induction of long-lived chromosome damage, as manifested by sister-chromatid exchange in lymphocytes of animals exposed to Mitomycin-C. Mutat. Res. 51:383-396.

Taylor, R. T., J. H. Carver, M. L. Hanna, and D. L. Wandres. 1979a. Platinum induced mutations to 8-azaguanine resistance in Chinese hamster ovary cells. Mutat. Res. 67:65-80.

Taylor, R. T., J. A. Happe, M. L. Hanna, and R. Wu. 1979b. Platinum tetrachloride: Mutagenicity and methylation with methylcobalamin. J. Environ. Sci. Health A14(2):87-109.

Thompson, L. H. 1979. Validation of conditions for efficient detection of HPRT and APRT mutations in suspension-cultured Chinese hamster ovary cells. Preprint UCRL-82111. Lawrence Livermore Laboratory, Livermore, California. Also: Mutat. Res. (in press).

Wyrobek, A. J., and R. Bruce. 1978. The induction of sperm-shape abnormalities in mice and humans. Chemical Mutagens, Vol. 5 (A. Hollaender, ed.), pp. 257-285. Plenum Press, New York.

Wyrobek, A. J., and B. L. Gledhill. 1978. Human semen assays for workplace monitoring. Proc. Workshop on Methodology for Assessing Reproductive Hazards in the Workplace, NIOSH. Preprint UCRL-81810. Lawrence Livermore Laboratory, Livermore, California.

8. EVALUATION OF POTENTIAL TOXICITY OF SYNTHETIC FUEL COMBUSTION PRODUCTS

D. L. Coffin
J. L. Huisingh

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

INTRODUCTION

In evaluating the potential health effects of synthetic fuels, it is necessary to test the fuels at every stage of extraction, refining, transportation, and end use at which a possibility exists of significant direct or indirect human contact. The production and subsequent refining of a synthetic crude oil from shale have presented a timely opportunity to examine combustion products. This report presents a brief overview of current and proposed studies of combustion products by investigators from EPA's Office of Research and Development at Research Triangle Park, North Carolina.

ONGOING AND PLANNED STUDIES

Studies are planned of the emissions which result from combusting synthetic fuel oil in commercial boilers. Comparisons to standard petroleum fuel oil emissions will seek possible differences in emitted products having direct toxicity (e.g. carcinogenicity or mutagenicity) or indirect effect (e.g., contribution of reactive hydrocarbons, nitrogen oxides, or other precursors to photochemical smog).

The mutagens and potential carcinogens associated with diesel combustion are of concern because of the high particle emission rate as compared to current gasoline vehicles. The minute particles emitted from diesel combustion consist of elemental carbon with adsorbed organics containing extractable mutagens (as indicated by Ames testing). Previous studies (Huisingh et al. 1978) showed that petroleum-derived diesel fuel combustion products (particles) possess significant mutagenic activity as measured by the Ames test. Furthermore, the characteristics of the fuel were observed to influence the Ames test results.

Underway is a joint EPA/Department of Transportation project to compare automotive combustion emissions from shale-oil-derived and petroleum-derived diesel fuels. This work employs the Diesel Fuel Marine refined by the Standard Oil Company of Ohio from the Paraho crude produced by Development Engineering, Inc. This fuel is to be compared for mutagenicity with standard petroleum-derived Diesel Fuel No. 2 obtained from local sources. As an analytical reference, these experiments will also include a No. 2 National Average (a fluid used for vehicle certification). Particles will be collected from a prototype test vehicle operated on a chassis dynomometer simulating the actual driving pattern of the Highway Fuel Economy Test cycle. Collection will be accomplished by filtration on Pollflex filters from a standard dilution tunnel. The filters will be extracted with dichloromethane for 48 h by the Soxhlet method and diluted with dimethyl sulfoxide for bioassay in the Ames test.

RESEARCH NEEDS

The type of information that is obtained in such studies is needed as quickly as possible in the hope of obviating, during extraction and refining operations, any problems that may be unique to synthetic fuels. In the present case, shale is considered a useful source for strait run middle distillates; as such, shale might prove a valuable substitute for petroleum for automotive diesel fuel. Information is needed not only on the currently available refined product (Diesel Fuel Marine) but also on the contribution

of mode of extraction and refining to the development of mutagens in the exhaust. Experiments are required to determine the influence of precursors in the fuel (regardless of the fuel's source) on the content of mutagenic compounds in the exhaust. Thus, basic experiments are needed not only on finished fuels but also on precursor fuels that have not been subjected to post-distillation treatment and, most importantly, on fuels in which the hydrocarbons have been deliberately altered in order to delineate the roles of specific fuel compound classes on mutagen synthesis by the diesel engine. Such a program, together with improvement of engine combustion efficiency, can likely lead to significant reduction in the content of potentially hazardous exhaust products.

REFERENCE

Huisingh, J., R. Bradow, R. Jungers, L. Claxton, R. Zweidinger, S. Tejada, J. Bumgarner, F. Duffield, M. Waters, V. F. Simmon, C. Hare, C. Rodriguez, and L. Snow. 1978. Application of bioassay to the characterization of diesel particle emissions. Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures (M. D. Waters, S. Nesnow, J. L. Huisingh, S. S. Sandhu, and L. Claxton, eds.), pp. 381-418. Plenum Press, New York.

* U.S. GOVERNMENT PRINTING OFFICE: 1981-757-064/0279