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Health Effects Research in Oil Shale Development

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Biology Division

HEALTH EFFECTS RESEARCH IN OIL SHALE DEVELOPMENT

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TABLE OF CONTENTS

	Page
PREFACE	ix
INTRODUCTION	xi
EXECUTIVE SUMMARY	xiii
SECTION 1: SHORT-TERM TESTING OF OIL SHALE MATERIALS	1
PART (A): MICROBIAL MUTAGENESIS ASSAYS	1
Bacterial Mutagenesis Assays	1
Yeast Mutagenesis Assay	8
Comparisons with Other Synfuels and Petroleum Crudes	8
Conclusions	11
PART (B): CYTOTOXICITY ASSAYS	12
Experimental Procedures	12
Experimental Results	12
Shale Oils	12
Comparative Research Materials (CRM)	13
Shale Oil Retort Water	15
PART (C): EMBRYOTOXICITY AND TERATOGENICITY	17
Aqueous Extracts of Three Oil Shale-Derived Materials	17
Aqueous Extracts of Comparative Research Materials	23
References (Section 1)	25
SECTION 2: ACUTE TOXICITY OF SELECTED CRUDE AND REFINED SHALE OIL- AND PETROLEUM-DERIVED SUBSTANCES	27
Introduction	27
Experimental Procedures	27
Substances Evaluated	27
Acute LD_{50} in Mice	28
Acute Dermal Toxicity in Rats	29
Delayed-Type Allergic Contact Hypersensitivity in Guinea Pigs	29
Primary Eye Irritation in Rabbits	30
Primary Skin Irritation in Rabbits	30
Histopathology in Mice	30
Lung Adenoma Assay in Strain A Mice	31
Results	31
Acute Toxicity in Mice and Rats	31
Delayed-Type Contact Hypersensitivity	34

TABLE OF CONTENTS (Cont'd.)

	Page
Eye and Skin Irritation in Rabbits	34
Histopathology	34
Lung Adenoma Assay	35
Discussion	42
References (Section 2)	43
SECTION 3: THE CHRONIC DERMAL TOXICITY OF PARAHO SHALE OIL AND SHALE OIL DISTILLATES	46
Introduction	46
Materials and Methods	46
Animal Exposures	46
Test Materials	46
Results	47
Skin Tumor Induction	47
Systemic Toxicity	47
Renal Toxicity	53
Summary and Conclusions	56
References (Section 3)	56

FIGURE LEGENDS

Figure Number	Section 1	Page
1	Sephadex LH-20 fractionation of shale oil coupled with mutagenicity testing. Specific mutagenic activities given were determined for the linear portions of dose response curves in the non-toxic dose range	4
2	Subfraction of ether soluble base fraction of shale oil using alumina and Sephadex LH-20 column chromatographics .	6
3	Forward mutation to canavanine resistance in S. cerevisiae induced by Paraho shale oil 4601: x, crude shale oil without activation; □, crude shale oil with phenobarbital-induced activation (rat liver preparation); +, ether-soluble basic fraction without activation; △, ether-soluble basic fraction with phenobarbital-induced activation (rat liver preparation).	9
4	Forward mutation to canavanine resistance in S. cerevisiae induced by hydrotreated shale oil 4602: x, HDT shale oil without activation; □, HDT shale oil with phenobarbital-induced activation (rat liver preparation); +, ether-soluble basic fraction without activation; △, ether-soluble basic fraction with phenobarbital-induced activation (rat liver preparation)	10
	Section 2	
1	Survival of mice given (A) 24 injections of crude shale oil 4601 or (B) hydrotreated shale oil 4602. (A): ●, 2.5 g/kg; ■, 1.25 g/kg; ▲, 0.5 g/kg; ○, corn oil. (B): ●, 16 g/kg; ■, 8 g/kg; ▲, 3.2 g/kg; ○, corn oil. Brackets indicate SE's.	39
2	Body weight of mice given 24 injections of crude shale oil 4601 or hydrotreated shale oil 4602. 4601: ○, 2.5 g/kg; ■, 1.25 g/kg; ▲, 0.5 g/kg. 4602: , 8 g/kg; △, 3.2 g/kg; ○, corn oil. Number of mice shown in parentheses. Brackets indicate SE's.	40

TABLE LEGENDS

Table Number	Introduction	Page
I	ORNL INVOLVEMENT - Toxicological Assessment - Paraho/ Sohio Refined Shale Oil	xi
	Section 1:	
A-I	Distribution of Mutagenic Activity in Fractions of *In Situ* Shale Oil	2
A-II	Subfractionation of Ether-Soluble Basic Fraction from Simulated *In Situ* Shale Oil	5
A-III	Salmonella Mutagenesis of Paraho Shale Oil	7
A-IV	Summary of Paraho-Sohio Shale Oil Work with Salmonella . .	8
A-V	Comparative Studies with Petroleum Crudes and Coal Derived Oils	11
B-I	Lethality of Water-Soluble Extracts of Selected Paraho Shale Oil Materials	13
B-II	Reproduction Impairment of Medium-Soluble Extracts of Selected Paraho Shale Oil Materials	14
B-III	Cytolethality of Aqueous Extracts of Three Crude Oils . . .	15
B-IV	Population Growth Impairment of Medium-Soluble Extracts of Three Crude Oils	16
C-I	Embryo Toxicity and Teratogenicity of Water Soluble Extracts of Estonian Generator Tar	19
C-II	Embryo Toxicity and Teratogenicity of Water Soluble Extracts of Paraho Crude Shale Oil	20
C-III	Embryo Toxicity and Teratogenicity of Water Soluble Extracts of Hydrotreated Paraho Shale Oil	21
C-IV	Comparative Embryo Toxicity and Teratogenesis of Aqueous Extracts of Estonian Generator Tar (4403), Paraho Crude Shale Oil (4601) and Hydrotreated Paraho Shale Oil (4602) .	22
C-V	Embryolethality of AEs of Three Crude Oils	24
C-VI	Teratogenesis of Aqueous Extracts of Three Crude Oils . . .	24
	Section 2:	
I	Acute Lethality Test in Mice: Shale Oil and Its Derivatives	32
II	Acute Lethality Test in Mice: Petroleums and Their Derivatives	33

TABLE LEGENDS (Cont'd.)

Table Number		Page
III	Weight Gain in Male and Female Rats Following Dermal Application of 2 g/kg of Shale Oil, Hydrotreated Shale Oil, or Hydrotreated Residue	35
IV	Delayed-Type Allergic Contact Hypersensitivity Test in Guinea Pigs	36
V	Primary Eye Irritation Test in Rabbits	37
VI	Primary Skin Irritation Test in Rabbits	38
VII	Mouse Lung Adenoma Assay: Shale Oil and Hydrotreated Shale Oil	41
VIII	Skin and Eye Toxicity of Petroleum-Based Substances	43
IX	Acute Oral Lethality Test of Selected Coal Conversion Products in BALB/c Male Mice	45
	Section 3:	
I	Skin Tumor Frequencies and Average Latency in C3H Mice Exposed to Paraho Process Shale Oil Before and After Upgrading	48
II	Skin Tumor Frequency and Average Latency in C3H Mice Exposed to Shale Oil Derived Commercial Distillates	49
III	Skin Tumor Frequency and Average Latency in C3H Mice Exposed to Petroleum Derived Commercial Distillates	50
IV-A	Mortality and Weight Comparisons for Shale Oil, Shale Oil Distillate and Petroleum Distillate in Female C3H Mice	51
IV-B	Mortality and Weight Comparisons for Shale Oil, Shale Oil Distillate and Petroleum Distillate in Male C3H Mice	52
V	Average Daily Water Consumption in Mice Exposed to Shale Oils and Distillates for a Minimum of 30 Weeks	54
VI	Urine Characteristics in Mice Undergoing Chronic Dermal Exposure to Middle Distillate	55

PREFACE

OIL SHALE ACTIVITIES

A multi-divisional effort (Life Sciences Synthetic Fuels Program, managed by K. E. Cowser) aimed at the integrated assessment of the health and environmental effects of various coal conversion and shale oil technologies is being carried out. Part of the Biology Division's efforts in the oil shale technologies are summarized below.

The biological work was, in many cases, carried out as a collaborative effort with the Analytical Chemistry Division. We specifically want to acknowledge M. R. Guerin, W. H. Griest, B. R. Clark, and C. H. Ho for their role in the fractionation and biopreparation of the materials. Samples examined in this study were supplied by the USEPA/USDOE Synfuels Research Materials Facility for generic research into the chemical and biological properties of petroleum substitutes.

Paraho/Sohio Shale Oil

The principal focus of the Paraho/Sohio Shale Oil project is the testing of primary effluents and products for potential effects on man. This portion of the evaluation of Paraho samples is concerned with questions of relative toxicities of process materials and refinery products.

The design of our approach to answer these questions in an expeditious and cost-effective manner involves a parallel, two-level program. Level one is cellular bioassays. These assays will accumulate baseline data on typical effluents and emissions and ascertain how the relative toxicity of major effluents and fractions thereof vary as a function of changes in process conditions. In addition, biological-effects studies using cellular assays will provide an essential data base for eventual determination of correlation with whole animal, acute, and chronic toxic effects.

Studies on short-term mutagenicity, cytotoxicity, embryotoxicity, and teratogenicity have identified the potentially hazardous materials. Chemical fractionation coupled with the bioassays has aided in isolating the specific components responsible for the biological damage.

Level two consists of the mammalian toxicity bioassays. These assays involve characterization of the acute, subacute, and chronic toxicity of primary process precursors and products. A study on acute mammalian toxicity

of selected Paraho samples was completed. Overall oral and dermal toxicity of shale oil and some of its derivatives was comparable to petroleum-derived products: all samples were only slightly toxic and were not irritants for skin or eyes. However, crude shale oil, hydrotreated shale oil, and hydrotreated residue produced delayed-type contact sensitization in guinea pigs. Data with the mouse lung adenoma assay suggest that crude shale oil has carcinogenic potential. Skin carcinogenicity has been obtained, in decreasing order of potency, for the crude shale oil, the residue after hydrotreatment and the hydrotreated shale oil. Systemic toxicity involving damage to the distal portion of the renal nephron has been noted 8 months after exposure to distillates from both shale oil and natural petroleum.

Early attention to the possibility of amelioration of the biological effects through upgrading of the crude oils has provided key information for the technologists.

INTRODUCTION

The developing oil shale industry is concerned with evaluating both short-term as well as long-term health effects associated with the technology. This task includes testing of primary effluents and products in various biological test systems from which risk posed to the shale oil industrial worker as well as consumers can be assessed. The Biology and Analytical Chemistry Divisions of Oak Ridge National Laboratory have been involved in such assessment studies with a suite of samples obtained from Paraho/Sohio/U.S. Navy. Table I illustrates various test samples and biological studies with these samples. The crude oil samples, the hydrotreated oil sample along with the fuel oils were subjected to extensive analysis in a variety of animal tests (mouse), genetic tests using microbes, cytotoxicity and embryotoxicity tests and eventual correlation to chemical constituents, determined by chemical analysis. Other test materials were analyzed in microbial mutagenesis assays that are rapid and cost-effective. Confirmatory studies were performed using the dermal carcinogenesis studies followed by chemical characterization.

Results obtained in these studies with this suite of samples are presented here.

Table I

ORNL INVOLVEMENT - Toxicological Assessment - Paraho/Sohio Refined Shale Oil

	Health Effects Bioassay									
Sample Type (Repository No.)	Acute oral	Acute dermal	Skin irritation	Eye irritation	Skin sensitization	Carcinogenesis dermal	Mutagenesis microbial	Teratogenesis	Cytotoxicity-embryotoxicity	Chemical characterization bioassay preparation
Crude shale oil (4601)	X	X	X	X	X	X	X	X	X	X
Hydrotreated shale oil (4602)	X	X	X	X	X	X	X	X	X	X
Gas feedstock (4603)										X
JP-5 precursor (4604)										X
JP-5 shale oil (4608)							X			
JP-5 petroleum (4614)							X			

Table I (Cont'd.)

ORNL INVOLVEMENT - Toxicological Assessment - Paraho/Sohio Refined Shale Oil

Sample Type (Repository No.)	Health Effects Bioassay									
	Acute oral	Acute dermal	Skin irritation	Eye irritation	Skin sensitization	Carcinogenesis dermal	Mutagenesis microbial	Teratogenesis	Cytotoxicity-embryotoxicity	Chemical characterization bioassay preparation
JP-8 precursor (4605)										X
JP-8 shale oil (4609)							X			X
JP-8 petroleum (4615)							X			X
DFM precursor (4606)										X
DFM shale oil (4610)							X			X
DFM petroleum (4616)							X			X
Acid sludge (4612)										X
Hydrotreated residue (4607)	X	X	X	X	X	X	X	X	X	X

EXECUTIVE SUMMARY

- Paraho/Sohio Shale Oil was found to be mutagenic in the Ames assay and confirmed in the yeast system.
- After chemical fractionation of the crude shale oil, it was found that the mutagenic activity was contributed by the organic constituents of the basic and neutral fractions.
- Hydrotreatment of the shale oil abolished the detectable mutagenic activity and also reduced the cytotoxicity as measured in cellular systems.
- Refined shale oil, jet fuel, and diesel fuel marine samples were not mutagenic.
- The samples rank for their mutagenic activity as coal oils > shale oil > natural petroleum crudes and only qualitatively agree with carcinogenic activity.
- Acute toxicity of Paraho Crude Shale Oil and its upgraded derivatives does not appear to be a problem of immediate concern.
- The data obtained in the lung adenoma bioassay suggest that Crude Shale Oil has tumorigenic potential.
- Paraho shale oil is carcinogenic in mouse skin.
- Hydrotreatment reduces but does not eliminate skin carcinogenicity and appreciable carcinogenic activity remains in the residue material.
- Kidney injury was noted following chronic dermal exposure to shale and petroleum derived middle distillates.

SECTION 1: SHORT-TERM TESTING OF OIL SHALE MATERIALS

PART (A): MICROBIAL MUTAGENESIS ASSAYS

The histidine reversion technique in *Salmonella typhimurium* developed by Ames et al. (1) and histidine reversion and canavanine resistance (forward mutation) in *Saccharomyces cerevisiae* (2) were used for mutagenicity determination. Metabolic activation was accomplished in these assays by incorporating mammalian (Aroclor induced rat liver) microsomal enzymes (1) into the assay.

Bacterial Mutagenesis Assays

The strain TA98 (*hisD* 3052, *uvrB*, *rfa*, *pkm* 101; obtained through the courtesy of Dr. B. N. Ames) (3) designed to detect frameshift mutations was used. The test material was dissolved in sterile dimethylsulfoxide and assayed for mutagenicity in a non-toxic dose range using the pour-plate incorporation method. The assay is quantitated with respect to dose and is represented as specific mutagenic activity (rev/mg).

An "in-situ" retorted shale oil sample was fractionated using the scheme described by Swain et al. (4) as modified by Bell et al. (5) and has been previously described (6). The neutral fraction has been subfractionated using a Florisil column. Reagent grade solvents and glass distilled water were used for extraction and chromatography.

Mutagenicity assays were performed with each primary fraction (acid, base, neutral) and the subfractionated neutral fractions. Specific mutagenic activities (rev/mg), weight distribution and weighted mutagenic activities (product of weight percent and specific activities) are given in Table A-I. The neutral fraction and the basic fraction contained most of the mutagenic activities.

Due to the fact that the Swain-Stedman fractionation scheme involves extreme conditions of chemical treatment, Jones et al. (7) have reported the use of liquid chromatographic separation using LH-20 Sephadex gel partitioning technique. Mutagenicity results obtained and the fractionation procedure are given in Figure 1. A total of approximately 300 rev/mg of the starting material was obtained by the summation of various fractions. The original material gave 233 rev/mg indicating a good biological recovery. Similar observation was also made for chemical recovery.

Table A-I

Distribution of Mutagenic Activity in Fractions of Simulated *in-situ* Shale Oil (LETC)

	No. Fraction[a]	Relative Weight % of Total	Specific Activity (Rev/mg)[b]	Weighted Activity (Rev/mg)
1	WA_I Weak $Acids_I$	0.05	185	>1
2	WA_E Weak $Acids_E$	1.23	52	1
3	SA_I Strong $Acids_I$	0.09	0	-
4	SA_E Strong $Acids_E$	0.26	159	>1
5	SA_W Strong $Acids_W$	0.55	160	1
6	B_{Ia} $Base_{Ia}$	0.20	1377	3
7	B_{Ib} $Base_{IB}$	0.26	800	2
8	B_E $Base_E$	7.11	952	68
9	B_W $Base_W$	0.28	223	1
	Neutral	86.66	112 (109)[c]	97
	Total	97.71		178
Neutral subfractions				
10	Hexane			
	A	58.69	40	23
	B	2.14	625	13
	C	1.27	750	10
11	Hexane/benzene			
	A	4.38	238	10
	B	1.89	340	6
	C	1.39	320	4
12	Benzene/ether			
	A	12.43	65	8
	B	2.19	142	3
	C	1.29	253	3
13	Methanol			
	A	15.12	179	27
	B	0.49	684	3
	C	0.93	263	2
	Subtotal	102.21		112

Table A-I (Footnotes) (Cont'd.)

Distribution of Mutagenic Activity in Fractions of Simulated *in-situ* Shale Oil

---

[a] I = insoluble (fractions a and b), E = ether-soluble, W = water soluble

[b] rev/mg = revertants/milligram, the number of histidine revertants from Salmonella strain TA98 by use of the plate assay with 2×10^8 bacteria per plate. Values are derived from the slope of the induction curve (with Aroclor induced S-9)

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

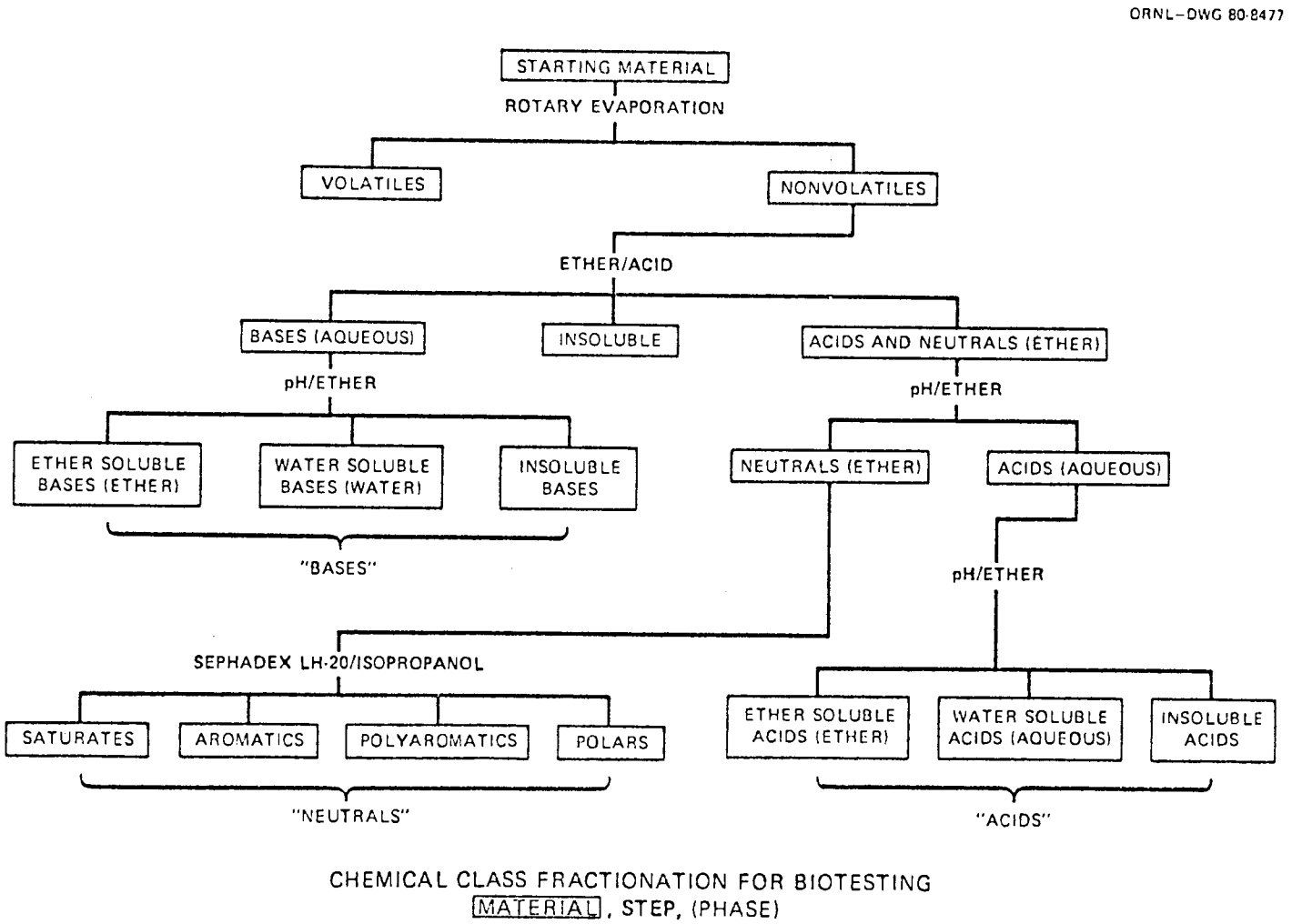

Figure 1. Sephadex LH-20 fractionation of shale oil coupled with mutagenicity testing. Specific mutagenic activities given were determined for the linear portions of dose response curves in the non-toxic dose range.

Since the basic subfraction contained significant mutagenic activity, the subfractionation technique developed by Guerin et al. (8) was used for the isolation of mutagenic agents in the ether soluble base fraction. Figure 2 illustrates the subfractionation of the ether soluble base fraction on a basic alumina column. The material is eluted with benzene followed by ethanol. Further isolation is achieved by eluting the ethanol eluate with isopropanol followed by acetone on a Sephadex LH-20 gel column. Table A-II illustrates the results obtained with an ether soluble base fraction from shale oil which show isolation of 90% of the mutagenic activity in the acetone fraction that accounts for 5% of the total weight of the starting fraction. Chemical analysis followed by mutagenesis assays have indicated aromatic amines (7) as the primary mutagenic constituents along with aza-arenes and other nitrogen containing polyaromatic hydrocarbons (9).

Table A-II

Subfractionation of Ether-Soluble Basic Fraction[a] from Simulated *in-situ* Shale Oil (LETC)

	Shale Oil	
	Wt. %	Rev/mg[a]
Basic fraction	100	2,500
Alumina		
Benzene	78	600
Ethanol	-	-
LH-20		
Isopropanol	12	0
Acetone	10	20,000

[a]Assayed with strain TA98 with Aroclor-induced preparation.

The Paraho Crude Shale Oil sample obtained from the Chemical Repository was subjected to chemical fractionation using the acid/base separation technique and the neutral fraction was subfractionated with Sephadex LH-20 gel chromatography into aliphatic and aromatic fractions of various ring sizes. The results obtained are given in Table A-III. The base fraction and the

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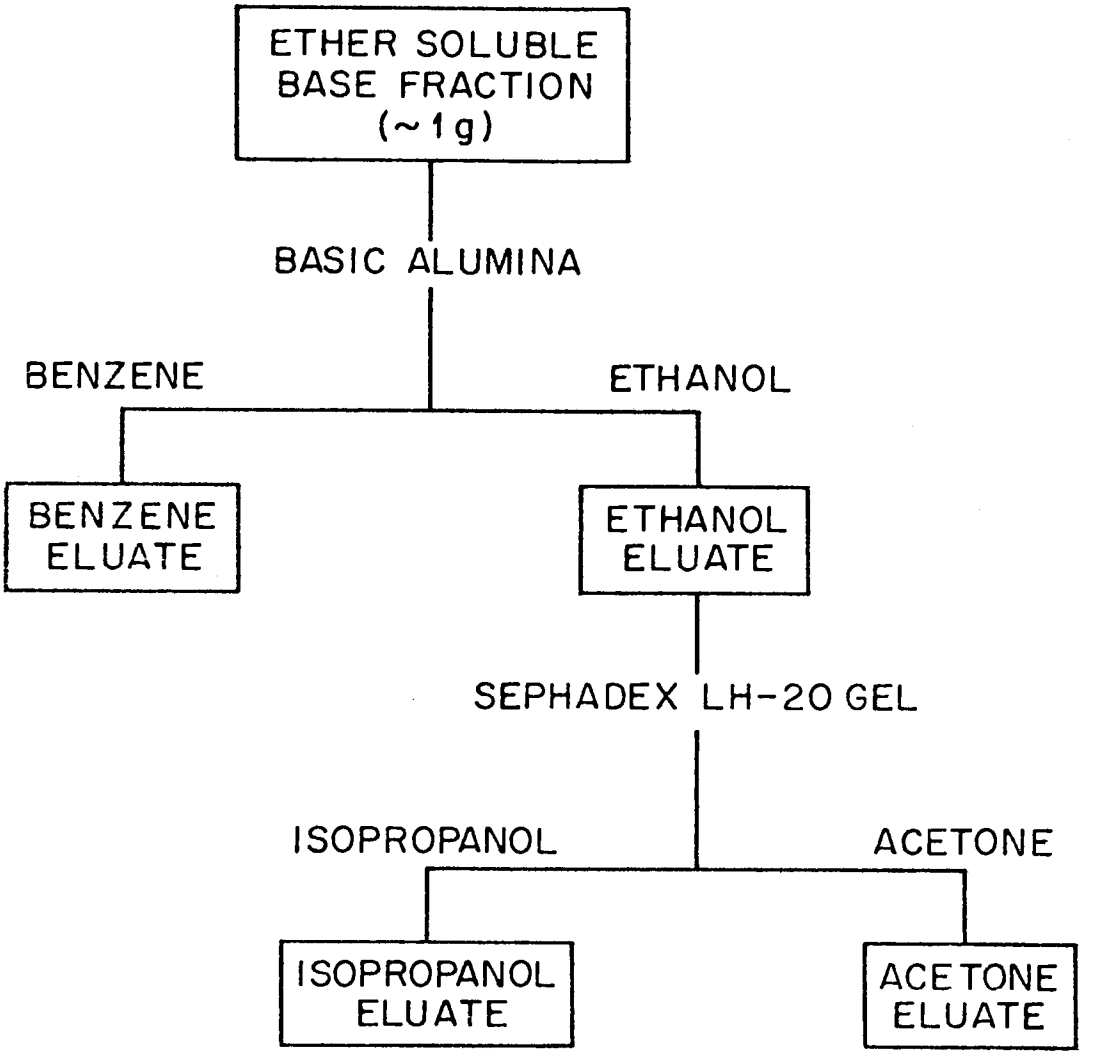

Figure 2. Subfraction of ether soluble base fraction of shale oil using alumina and Sephadex LH-20 column chromatographics.

Table A-III

Salmonella Mutagenesis of Paraho Shale Oil (4601)

Fractions	% Weight	Specific Activity (Rev/mg)	Weighted Activity (Rev/mg)
1. Volatiles	0.26	0	0
2. Et_2O sol. Acids	0.41	0	0
3. Et_2O sol. Bases	3.32	8,000	265
4. Acid ppt.	0.34	0	0
5. Base ppt.	0.50	141	1
6. Total Neutral	95.36	140	133 (116)[a]
a. Aliphatic	55.61	20	11
b. Mono- + Diarom.	27.37	0	0
c. Polyaromatic	6.95	1,600	111
d. Residue	2.15	0	0
		Total:	122
Total of Base Neutral Fractions:			398 (382)[a]
ORIGINAL	-	500	500

[a]Obtained by the summation of subfractions

neutral fraction were the major contributors of the mutagenic activity. By the summation of mutagenic activities of subfractions a value of 382 rev/mg of the original starting material was obtained. A suite of samples (ORNL repository numbers 4602-4616) from Paraho/Sohio refinery were tested (both raw samples and subfractions) for mutagenicity and the results are given in Table A-IV. The hydrotreated shale oil and its residue from fractionation were not mutagenic in the bioassay. The refined oil samples such as JP-5, JP-8 and DFM product as their precursors were not mutagenic in the Ames assays. Two petroleum equivalents from JP-5 and DFM failed to show mutagenic activities. These results were originally presented at the Shale Oil Symposium held in Gatlinburg, Tennessee, June 1980 (10).

Table A-IV

Summary of Paraho-Sohio Shale Oil Work With Salmonella System

ORNL Repository No.	Sample Description	Mutagenic Activity (Rev/mg)
4601	Crude Shale Oil	382
4602	Hydrotreated Shale Oil	0
4604	JP-5 Precursor	0
4605	JP-8 Precursor	0
4606	DFM Precursor	0
4607	Hydrotreated Residue	0
4608	JP-5 Product	0
4609	JP-8 Product	0
4610	DFM Product	0
4614	JP-5 Petroleum	0
4616	DFM Petroleum	0

Yeast Mutagenesis Assays

The results obtained with Salmonella were verified by using a multipurpose stock of *Saccharomyces cerevisiae*; strain XL7-10B has the genotype $\alpha\ \rho^+$ CAN1 *his*1-7 *lys*1-1 *ura*1. Forward mutation was monitored by selection of canavanine resistant mutants (CAN → *can*) (12,13,14,15); base-pair substitutions were detected by selection of prototrophic revertants of the missense marker *his*1-7 (his → HIS) (16,17). The data obtained with this strain are presented in Figures 3 and 4 and show that the crude shale oil was mutagenic and the ether soluble basic fraction also contained significant mutagenic activity. Both the canavanine marker and the histidine marker were affected. Some mutagenic constituents of shale oil are direct-acting, and the overall activity is enhanced by metabolic activation (Figure 3). The neutral polyaromatic fraction did not appear to be mutagenic to yeast (not shown). The hydrotreated sample (#4602) failed to show any mutagenic activity in either the raw sample or the ether-soluble base fraction (Figure 4; see also reference 11).

Comparison with Other Synfuels and Petroleum Crudes

Table A-V quantitatively compares the mutagenesis results obtained with shale, coal, and petroleum derived oils in the Salmonella assay.

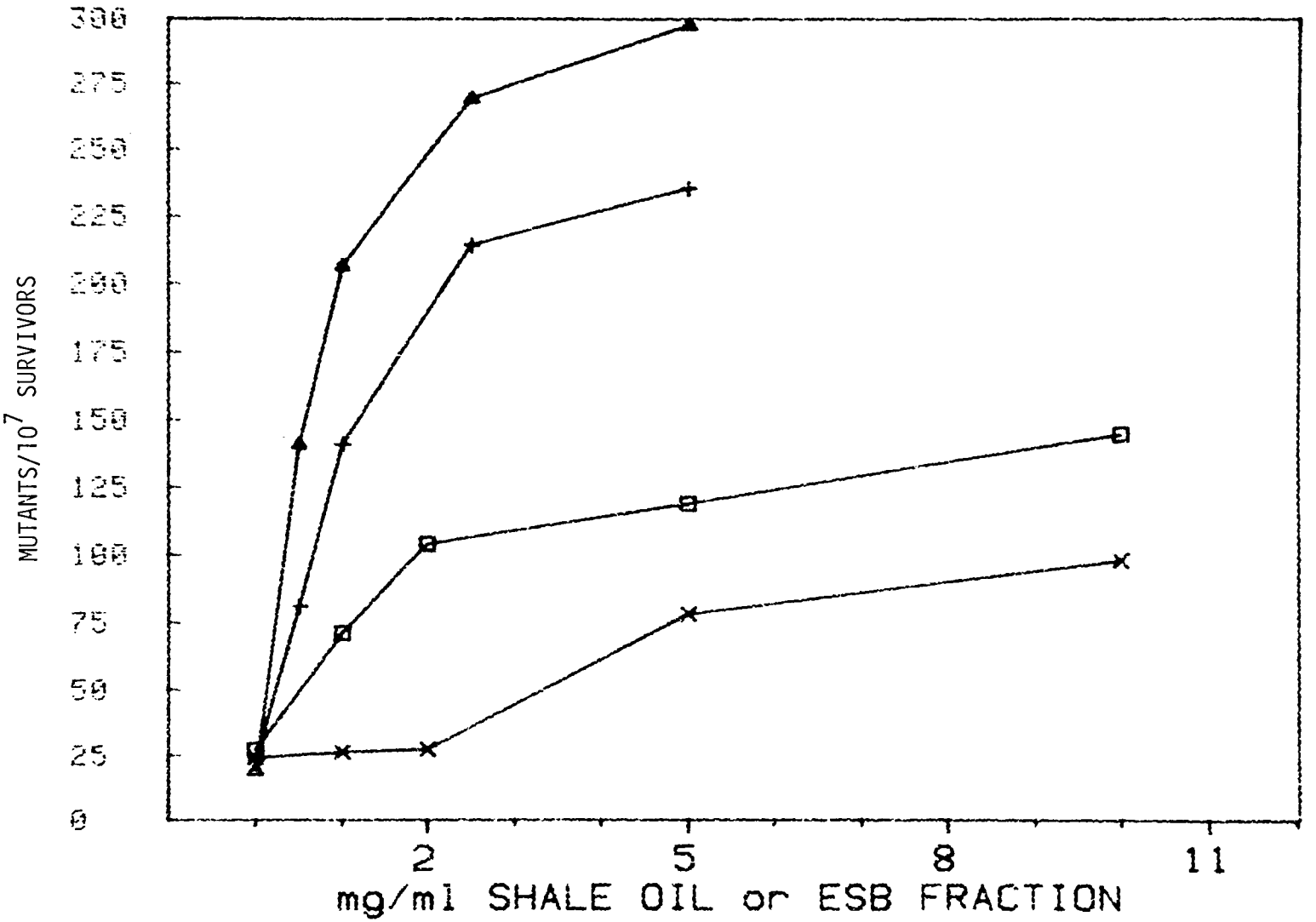

Figure 3. Forward mutation to canavanine resistance in S. cerevisiae induced by Paraho shale oil 4601: x, crude shale oil without activation; ◻, crude shale oil with phenobarbital-induced activation (rat liver preparation); +, ether-soluble basic fraction without activation; ▲, ether-soluble basic fraction with phenobarbital-induced activation (rat liver preparation).

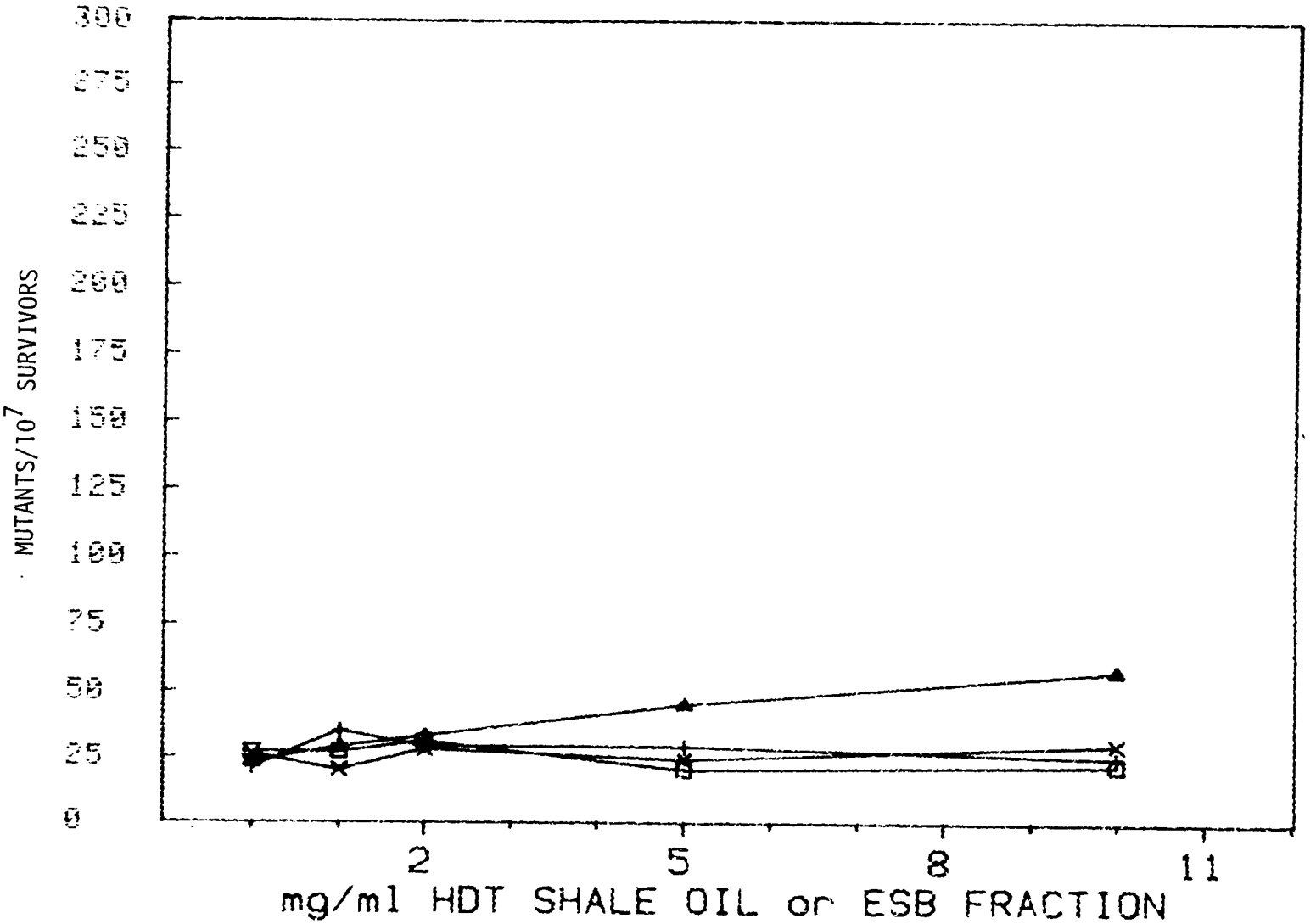

Figure 4. Forward mutation to canavanine resistance in S. cerevisiae induced by hydrotreated shale oil 4602: x, HDT shale oil without activation; □, HDT shale oil with phenobarbital-induced activation (rat liver preparation); +, ether-soluble basic fraction without activation; △, ether-soluble basic fraction with phenobarbital-induced activation (rat liver preparation).

Table A-V

Comparative Studies[a] with Petroleum Crudes and Coal Derived Oils

Repository No.	Sample	% Distribution Acid	Base	Neutral	Mutagenicity (Rev/mg)
5301	Wilmington Crude Oil	0	0	100	5
5305	Recluse Crude Oil	0	0	100	6
1701	SRC-II Fuel Oil	0	36	64	959
1601	H-Coal Distillate	0	41	59	311
1604	H-Coal, HDT (high)	0	0	0	0
4101	Laramie Shale Oil	0	56	44	146
4601	Paraho-Sohio Shale Oil	0	69	30	382
4602	Paraho-Sohio Shale Oil, HDT	0	0	0	0

[a]Salmonella histidine reversion (Ames assay).

Natural petroleum crudes are the least mutagenic with all the activity remaining in the neutral fractions. A coal derived fuel oil (SRC-II) was highly mutagenic, with the neutral fraction being the major contributor. Another oil sample, a coal hydroliquefaction product oil (H-Coal) was also mutagenic, again most of the activity remaining in the neutral fraction. Hydrotreatment of this oil has eliminated the mutagenic activity, similar to hydrotreated shale oil. Both the Paraho/Sohio Crude Shale Oil and Laramie Shale Oil were mutagenic with the basic fractions being the major contributor of mutagenic activity.

Conclusions

1. Paraho/Sohio Shale Oil was found to be mutagenic in the Ames assay when assayed with the frameshift strain TA98 and incorporating metabolic activation with rat liver homogenates (Aroclor induced S-9).

2. The mutagenic activity was contributed by the organic constituents of the basic and neutral fractions.

3. Hydrotreatment of the shale oil abolished the mutagenic activity.

4. Results obtained in the yeast assay supported these observations.

5. HDT shale oil, jet fuels, and diesel fuel marine samples were not mutagenic.

6. The samples rank for their mutagenic activity as coal oils > shale oil > natural petroleum crudes.

PART (B): CYTOTOXICITY ASSAYS

Short-term static toxicity testing has been performed to examine the effects of soluble extracts of selected Standard Oil of Ohio-refined Paraho shale oil materials on *Tetrahymena pyriformis*. *Tetrahymena* is a large motile ciliate which provides a rapid and inexpensive test system. The Paraho shale oil materials tested include crude shale oil, hydrotreated shale oil, hydrotreated residue (No. 6 fuel oil), and diesel fuel marine product. In addition, a conventional, petroleum-derived marine diesel fuel was assayed for comparison. Results with an aqueous process waste, retort water from the Paraho facility, are also presented (see also ref. 19).

Experimental Procedures

Suspensions of *Tetrahymena pyriformis* were grown axenically at 28°C in a semidefined proteose-peptone medium. Near stationary-growth-phase cells were harvested by centrifugation prior to being resuspended in 5-ml aliquots of various concentrations of water-soluble extracts of the test samples. The EPA-suggested four-salt solution was used to simulate soft water. The concentration required to elicit 100% cell mortality within 24 hr (24-hr LC100) was determined with the aid of phase-contrast microscopy.

Reproduction impairment of *Tetrahymena* was studied spectrophotometrically. Test cultures, in 10-ml aliquots of each of the various concentrations of medium-soluble extracts of each of the test samples, were grown at 28°C in 2.5 x 15-cm glass tubes after inoculation with 0.2 ml of cell suspension. The concentration responses were examined by least-squares linear regression where Y is 72-hr absorbance and X is concentration. The concentration which inhibits 50% growth (72-hr IGC 50) and 95% confidence limits were calculated.

Soluble extracts were prepared by placing 30 ml of soft water or 30 ml of proteose-peptone medium with 5 ml of sample in a 60-ml separatory funnel. The mixture was shaken for 30 minutes on a reciprocal shaker (208, 12-mm strokes/minute). The mixture was allowed to stand 30 minutes after shaking prior to the phases being separated.

Experimental Results

Shale Oils

Data for the 24-hr LC100 are summarized in Table B-I. The crude shale oil extract is the most toxic material tested. Nine percent extract causes

Table B-I

Lethality of Water-Soluble Extracts of Selected Paraho Shale Oil Materials[a]

Sample	ORNL Ref. No.	Sohio Ref. No.	pH	24-Hr LC100
Crude shale	4601	C50-556	6.9	9%
HDT-Shale Oil	4602	C5HTSO-554	6.9	100%
HDT-Shale Oil Residue	4607	HTR-555	7.1	100%
Shale Oil Derived Diesel Fuel Marine	4610	FIN DFM	7.6	100%
Conv. petro. derived Diesel Fuel Marine	4616	---	7.6	100%

[a]Special Notice. These samples are intended for generic and scoping research only at this time and are not intended to define or compare specific processes in the absence of process operating conditions and detailed sampling and storage histories.

100% cell mortality within 24 hr. Eight percent causes 90 + % cell death while 6% causes 50% death. Concentrations as low as 2% cause a markedly reduced mortality. Hydrotreatment drastically reduces the toxicity of the shale oil extract. One-hundred percent extract has no effect on cell shape and only slightly reduced mortality when compared to controls. Neither the hydrotreated shale oil residue (No. 6 fuel oil) nor either of the diesel fuel marine products show any acute lethal effects.

Data for the 72-hr reproduction impairment studies are presented in Table B-II. The crude shale oil extract and hydrotreated shale oil extract are the only samples to elicit a response; the crude shale oil extract is three times more toxic than its hydrotreated counterpart. This reduction in toxicity with hydrotreatment has been observed with several coal conversion products tested previously. These results must be tempered by the fact that the shale oils, and especially the shale oil residue, are highly viscous at room temperature and the 30-minute, aggradation-extraction period may not provide as uniform a mixing as with other samples.

Comparative Research Materials (CRM)

Studies have been undertaken in which the cytotoxic effects of three organic liquid CRMs on *Tetrahymena pyriformis* have been monitored. The

Table B-II

Reproduction Impairment of Medium-Soluble Extracts of Selected Paraho Shale Oil Materials

Sample	Regression Equation	Correlation Coefficient	IGC50[a] (%)
Crude Shale Oil	Y = 0.024X + 0.483	-0.938	13.9 (5.8 - 33.2)
HDT-Shale Oil	Y = 0.003X + 0.298	-0.983	45.4 (13.1 - 152.5)
HDT-Shale Oil Residue	---	---	100% No Effect
Shale Oil Derived Diesel Fuel Marine	---	---	100% No Effect
Conv. Petro. Derived Diesel Fuel Marine	---	---	100% No Effect

[a]95% confidence limits in parentheses

three CRMs are a coal-derived liquid (CRM-1), a crude shale oil (CRM-2), and a petroleum crude oil (CRM-3). Aqueous and medium extracts were generated by slow stirring oil and aqueous mileux in a 1:8 ratio for 16 hr. Appropriate dilutions were made prior to short-term static testing for lethality and population growth impairment.

Mortality was determined by cell counting with the aid of dark-field microscopy. The concentration response in EPA "soft" four-salt test solutions were examined by least-squares linear regression where Y is the number of viable cells per 10 μl following 24 hr of exposure and X is the concentration as percent extract. Similarly, concentration levels that alter population growth monitored spectrophotometrically at 540 nm were examined by linear regression where Y is the percent control 48-hr absorbance and X is the concentration as percent extract.

Data for lethality are summarized in Table B-III. The extract of the coal-derived liquid is the most toxic of the three CRMs assayed. Five percent extract causes 100% cell mortality within 3 hr exposure. Four percent causes

Table B-III

Cytolethality of Aqueous Extracts of Three Crude Oils

Sample	Regression Equation	Correlation Coefficient	24-Hr LC_{50} (%)[a]	24-Hr LC_{100} (%)[a]
CRM-1 (Coal-Derived Liquid)	$Y = -11.000X + 45.30$	-0.975	2.62	5.0
CRM-2 (Crude Shale Oil)	$Y = -2.725X + 37.88$	-0.952	7.85	12.0
CRM-3 (Petroleum Crude)			100% Extract Had No Effect	

[a]Lethal concentration

95 + % cell death within 24 hr. On the other hand, 2% causes only a slight reduction in ciliary activity while 1% elicits no observed effect. The extract of the crude shale oil is half as toxic as that from CRM-1 with 12% causing 100% cell death within 3 hr. Six percent CRM-2 extract only slightly decreases cell numbers, whereas 4% elicits no observable effect. The petroleum crude oil is the least toxic CRM tested. One hundred percent CRM-3 extract causes only a slight reduction in cell motility and no cell death.

Data for the 48-hr population growth impairment studies are presented in Table B-IV. Again only the CRM-1 and CRM-2 exhibit much adverse effect. The no-observed effects concentration is 0.5%, 1.0%, and 60.0% for the CRM-1, CRM-2, and CRM-3, respectively.

Shale Oil Retort Water

Shale oil retort water is obtained by centrifuging the oil/water emulsion produced by oil shale retorting. The ciliate *Tetrahymena pyriformis* (originally published in ref. 19) was exposed to retort water; 2, 1, and 0.5% initially increased motility; longer exposures decreased motility. Three, 4, and 5% decreased motility. Cell lysis was directly related to concentration; after 24 hr, population densities were 0, 10, and 25% of controls for 2, 1, and 0.5% retort water, respectively. O_2 consumption paralleled the motility pattern in that at lower concentrations it increased initially but decreased with extended exposures; at higher concentrations it decreased rapidly. The most striking cytological alteration of cells exposed to the toxicant occurred

Table B-IV

Population Growth Impairment of Medium-Soluble Extracts of Three Crude Oils

Sample	Regression Equation	Correlation Coefficient	96 Hr LC_{50} (%)
CRM-1 (Coal-Derived Liquid)	$Y = -30.701X + 103.9$	-0.967	1.76
CRM-2 (Crude Shale Oil)	$Y = -8.147X + 102.7$	-0.963	6.47
CRM-3 (Petroleum Crude)	$Y = -0.999X + 163.8$	-0.903	113.88[a]

[a]33% growth inhibition, 97% extract

in the membranes; alterations of mucocysts and glycogen content were also observed, but mitochondrial changes were not. Population growth was affected at much lower concentrations than the other test indices. The growth of test populations plateaued at values inversely related to concentration; concentrations <0.4% had no effect on growth rate.

The data on cytotoxicity of shale oil retort water strongly suggests that at least some of the toxic effects reflect membrane damage, thus partitioning of the toxicant between hydrophobic and hydrophilic components of the membrane becomes important for understanding modes of toxicity. The limited effects of the major organic components of the retort water — straight-chain carboxylic acids and amides (20) — suggest that they contribute little to toxicity. Some analyses of shale oil retort waters are available. Gas chromatography of the shale oil retort water sample used in this study has identified straight-chain carboxylic acids of from 2 to 10 carbon atoms in length as the major organic components (85% of total organic content by weight). Phenols, the major organic components of product waters from coal-conversion processes, account for only 3% of the total organic content of shale oil retort water. Both are considered easily biodegradable by treatment plants, and we have found that concentrations of organic acids up to 20X that in the retort water are required to affect cells in a manner similar to 5% retort water. The amides are even less toxic. Thus, we feel that the toxicity of retort water is caused by other, perhaps inorganic, components.

Elemental analyses of oil shale and oil shale products have been made although standard reference materials and accurate analyses of all possible

important elements are not yet available. Furthermore, the elemental content may vary depending on the source of shale oil used and on the retorting parameters employed. Nonetheless, available data (B. Clark, personal communication) reveal the presence of an array of elements, including relatively high concentrations of tin, arsenic, sodium, fluorine, magnesium, bromine, selenium, and chlorine. The sample used in our study contained 188 mg/liter sodium, 33.4 g/liter bicarbonate, 14.9 g/liter carbonate, 2.8 g/liter chloride, 1.34 g/liter sulfate, 2.05 g/liter thiosulfate, and 5 g/liter DOC (dissolved organic carbon) (B. Clark, personal communication). A complete elemental analysis of this specific sample is not available, but it is known that samples of similar origin contain up to 5% inorganic components. Thus, the toxicity of retort water may be due to high concentrations of inorganic material, but confirmation must await further detailed analyses and/or comparative toxicity tests of the variety of elements known to be present in water from oil shale retorting processes.

Since the completion of this investigation Fox et al. (21) have characterized the oil shale retort water from the Laramie Energy Technology Center Site-9 *in situ* retort experiment and have found it to have a low total organic carbon content but high ammonia and inorganic sulfur concentrations. Bergman et al. (22) have corroborated the findings of these studies. They report a 96-hr LC50 for rainbow trout and fathead minnow of approximately 0.5% retort water. Similarly they note the 48-hr EC50 for *Daphnia* is 0.5% retort water and conclude that the principal toxic constituents in the acute tests were inorganic.

PART (C): EMBRYOTOXICITY AND TERATOGENICITY

Aqueous Extracts of Three Oil Shale-Derived Materials

The toxic and teratogenic potential of three oil shale derived materials has been examined using an amphibian teratogenesis assay model (ATAM) (*Xenopus laevis*).

Aqueous extracts were prepared by mixing 1 part organic material with 8 parts water for 16 hours. Replicates of 50 embryos were placed in graded concentrations of each extract and tested under acute static conditions. Following 96 hours of exposure, data were collected concerning the number of survivors and abnormal embryos, stage of their development, and their length, motility and pigmentation. Data on mortality and abnormality were analyzed by linear regression. Previous work has shown synthetic fossil energy related

materials to be highly toxic and teratogenic in the ATAM. The data presented here confirms and extends these findings.

The embryo toxicity and teratogenicity data for the Estonian generator tar (ORNL #4403), Paraho crude shale oil (ORNL #4601), and hydrotreated Paraho shale oil (ORNL #4602) are summarized in Tables C-I, C-II, and C-III, respectively. Plots of the mortality vs. concentration and abnormals/survivors vs. concentration data reveal direct linear relationships.

The linear regression models, calculated 96 hr LC_{50} and 96 hr EC_{50} (concentration necessary to cause abnormalities in 50% of survivors) values and 95% confidence limits are presented in Table C-IV. Aqueous extracts of the Estonian tar are 4 to 5 times more embryo lethal than those derived from Paraho shale crude. Hydrotreatment reduces the toxicity of the Paraho shale oil by a factor of nine.

Due to their differences in toxicity the three shale oil samples are not directly comparable, but each sample adversely affects mean length, stage of development, pigmentation and motility (see Tables C-I—C-III. Malformations appear to be sample specific and concentration dependent. A 96-hr control embryo presents a streamlined profile with compact viscera and smooth epithelium. Embryos exposed to aqueous extracts of generator tar reveal a general swelling of the facial and viscera region and a series of small epidermal blisters along the dorsal and ventral edge of the tail region. In addition, abnormalities associated with gut development (i.e., coiling) are apparent. Aspects of malformation in embryos exposed to aqueous extracts of untreated Paraho crude include severe dorsal tail flexion, optic and gut abnormalities, as well as slight general edema with few epidermal blisters. Developmental abnormalities of embryos exposed to aqueous extracts of hydrotreated Paraho shale oil are limited to general edema and gut development. While the Estonian and hydrotreated samples cause more severe edema and blisters, two characteristics associated with acute toxicity, the untreated Paraho sample causes a greater number of truly irreversible terata. In summary, aqueous extracts of Estonian generator tar and two Paraho shale oil crudes have been compared on the basis of their embryotoxic and teratogenic effects. While the generator tar is 4-5 times more toxic than untreated shale oil crude the latter causes a greater number and more severe malformations. Hydrotreatment sharply decreases the toxicity and teratogenicity of the Paraho shale oil.

Table C-I

Embryo Toxicity and Teratogenicity of Water Soluble Extracts of Estonian Generator Tar

Concentration (%)	0	0.5	1.0	1.5	2.0	2.5
Percent Dead	4.8	15.2	10.4	16.0	64.0	94.0
Percent Abnormal/Survivors	14.2	3.8	58.0	100.0	100.0	100.0
Developmental Stage[a]	47	47	45	-	-	-
Mean Length (mm)[b]	9.32	9.06	8.00	-	-	-
Pigmentation[c]	+4	+4	+3	+3	+2	+1
Motility[c]	+4	+4	+2	+1	0	0

[a]Of normal embryos according to Nieuwkoop and Faber (1975)

[b]Of normal embryos

[c]Quantitative index based on control embryos

Table C-II

Embryo Toxicity and Teratogenicity of Water Soluble Extracts of Paraho Crude Shale Oil

Concentration (%)	0	5	7.5	10	12.5
Percent Dead	2	10	39	71	100
Percent Abnormal/Survivors	5	77	100	100	--
Developmental Stage[a]	47	45	--	--	--
Mean Length (mm)[b]	8.83	8.20	--	--	--
Pigmentation[c]	+4	+4	+3	+2	--
Motility[c]	+4	+3	+3	+2	--

[a]Of normal embryos according to Nieuwkoop and Faber (1975)

[b]Of normal embryos

[c]Quantitative index based on control embryos

Table C-III

Embryo Toxicity and Teratogenicity of Water Soluble Extracts of Hydrotreated Paraho Shale Oil

Concentration (%)	0	40	50	60	70	80
Percent Dead	9	6	6	40	61	79
Percent Abnormal/Survivors	8	0	56	100	100	100
Developmental Stage[a]	47	47	46	--	--	--
Mean Length (mm)[b]	8.58	ND[d]	8.26	--	--	--
Pigmentation[c]	+4	+4	+4	_3	+1	+1
Motility[c]	+4	+4	+4	+2	0	0

[a]Of normal embryos according to Nieuwkoop and Faber (1975)

[b]Of normal embryos

[c]Quantitative index based on control embryos

[d]Not determined

Table C-IV

Comparative Embryo Toxicity and Teratogenicity of Aqueous Extracts of Estonian Generator Tar (4403), Paraho Crude Shale Oil (4601), and Hydrotreated Paraho Shale Oil (4602)

ORNL Sample	Regression Equation	Correlation Coefficient	96-Hr EC_{50} (%)	96-Hr LC_{50} (%)
4403	Y = 51.44X - 46.00	0.964	--	18.7
4403	Y = 96.20X - 42.27	0.997	0.96	--
4601	Y - 7.94X - 10.78	0.937	--	7.65
4601	Y = 12.91X - 6.85	0.995	3.34	--
4602	Y = 2.27X - 101.70	0.992	--	66.83
4602	Y = 4.70X - 181.00	0.999	49.15	--

Aqueous Extracts of Comparative Research Materials

Studies are near completion in which the effects of aqueous extracts (AEs) of three CRMs on early embryos of *Xenopus laevis* are being tested. The three CRMs are a coal-derived liquid (CRM-1), a crude shale oil (CRM-2), and a petroleum crude oil (CRM-3). Aqueous extracts are prepared by placing the individual CRMs and dechlorinated water in a flask in a ratio of 1:8. The flask contents are then slow-stirred for 16 hr prior to phase separation. For short-term static testing freshly prepared extracts are appropriately diluted with dechlorinated water to form a graded concentration series. Replicate groups of 50 embryos each are placed in 200 ml aliquots of test solution in 125- x 60-mm glass bowls that are covered to minimize evaporation. At representative time points, embryos are examined with the aid of a dissecting microscope, and dead specimens are removed. Following 96 hr of exposure, information concerning mortality, number and type of abnormalities, stage of development, size, and relative motility and pigmentation are recorded. Mortality and abnormals and survivors are analysed by linear regression.

Mortality data are summarized in Table C-V. The extract of CRM-1 is nine times more toxic than that of CRM-2 which, in turn, is four times more toxic than that of CRM-3. Teratogenic data are summarized in Table C-VI. The CRM-2 causes the largest proportion of nonlethal abnormalities, as evidenced by the ratio of LC_{50} to effective concentration (EC_{50}) for each CRM. The predominant abnormalities include: tail flexure, pericardial edema, and optic abnormalities. Embryo length, stage of development, and motility and pigmentation also appear to be inversely related to extract concentration, especially for CRM-1 and CRM-2. (L. J. Sutor, GLCA/ACM Science Semester Research Participant, Colorado College, Colorado Springs, Colorado, contributed to these studies.)

The results from microbial mutagenesis, cytotoxicity, embryotoxicity, and teratogenicity testing only act as a preliminary measure of potential hazard and serve to rapidly identify specific components. Whole-animal testing is still necessary to begin understanding comparative risk for man. Test development and validation across systems needs further work.

Table C-V

Embryolethality of Aqueous Extracts of Three Crude Oils

Sample	Toxicity Regression Equation	Correlation Coefficient	96-Hr LC_{50} (%)	96-Hr LC_{100} (%)
CRM-1	Y = 58.46X - 42.28	0.985	1.58	2.43
CRM-2	Y = 6.59X - 34.10	0.993	12.77	20.36
CRM-3	Y = 2.35X - 60.16	0.931	46.80	68.04

Table C-VI

Teratogenicity of Aqueous Extracts of Three Crude Oils

Sample	Teratogenesis Regression Equation	Correlation Coefficient	96-Hr EC_{50} (%)	96-Hr EC_0 (%)
CRM-1	Y = 51.78X + 1.46	0.987	0.94	0.25
CRM-2	Y = 6.41X + 4.74	0.975	7.06	2.00
CRM-3	Y = 2.32X - 46.26	0.986	41.53	19.94

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SECTION 2: ACUTE TOXICITY OF SELECTED CRUDE AND REFINED SHALE OIL- AND PETROLEUM-DERIVED SUBSTANCES*

INTRODUCTION

To help evaluate and predict hazards of substances to which humans may be exposed in the workplace, several kinds of tests with laboratory animals can be used. These tests are usually designed to provide information on overall toxicity of a given compound and to detect its potential to cause untoward effects.

It was the objective of the present study to obtain general information on the toxicity of selected samples of Crude Paraho Shale Oil and some of its derivatives, some crude petroleums, and three refined petroleum products. Five tests were used to determine the acute toxicity of these substances: acute lethality in mice following oral or intraperitoneal administration of a single dose; acute dermal toxicity of a single dose in rats; delayed-type allergic contact hypersensitivity in guinea pigs; primary eye irritation and primary skin irritation of a single dose in rabbits. In addition, we have examined histopathologic changes induced in mice following intraperitoneal (ip) injection of a single large dose of Crude Shale Oil and two of its hydrotreated derivatives. Studies also have been initiated to examine the tumor inducing potential of selected samples in the mouse lung adenoma bioassay. The present report describes our findings and shows that all compounds tested have very low or no acute toxic effects in laboratory animals.

EXPERIMENTAL PROCEDURES**

Substances Evaluated

Bulk samples of the materials were received from the Chemical Repository. Viscous samples were thoroughly mixed by shaking after being warmed in a water

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**The test methods used are based on those described in Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington, DC, 1977. Acute toxicity, pp. 10-17; dermal toxicity, pp. 23-26; delayed-type sensitivity, pp. 36-39; eye irritation, pp. 123-124; skin irritation, pp. 28-31.

bath at 70-80°F. Where feasible, samples were applied undiluted. For the experiments in which it was necessary to administer graded doses, measured portions were diluted with corn oil (laboratory grade, Fisher Scientific) to provide the desired final concentrations; the density of all substances was assumed to be 1.0.

The following substances were studied:

ORNL Repository No.	Name
Shale Oil and Its Derivatives	
4601	Crude Shale Oil
4602	Hydrotreated Shale Oil
4607	Hydrotreated Residue
4608	JP-5 Product (jet fuel)
4609	JP-8 Product (jet fuel)
4610	Diesel Fuel Marine Product
Petroleum and Its Derivatives	
5107	Mixed Petroleum Crudes
5301	Wilmington Crude Oil
5305	Recluse Crude Oil
4614	JP-5 Product (jet fuel)
4615	JP-8 Product (jet fuel)
4616	Diesel Fuel Marine Product

Acute LD_{50} in Mice

The objective of this test was to determine acute toxicity following a single oral or ip administration of a substance. The animals used were BALB/c male or female mice that weighed 20-25 g.

Mice were randomly grouped six per cage and were fasted for 18 hr before administration of a substance. For oral administration a ball-tipped needle (20 gauge) fitted to a 1-ml syringe was used. The administration volume was 10 μl/g body weight except when a dose of 16 g/kg was delivered; in this case 20 μl/g body weight of a solution containing 0.8 ml/ml of the test agent was administered. The doses were 2, 4, 8, and 16 g/kg. For ip injections, a 1-ml syringe fitted with a 22-gauge needle was used. The injection volume was 10 μl/g body weight, and doses were 1, 2, 4, and 8 g/kg. Additional doses were tested when necessary. Corn oil was the vehicle used for both routes of administration.

Animals were returned to their cages with free access to food and water and were observed for signs of toxicity. The number of survivors was recorded

daily. Two weeks after administration of the substances, the surviving mice were killed. The LD_{50} was calculated from the number of 14-day survivors according to the procedures of Weil (1).

Acute Dermal Toxicity in Rats

The objective was to determine the acute toxicity of a substance following a single application to the skin. The animals used for this test were albino male (300-400 g) and female (200-250 g) rats. Each substance was tested on five or six animals of each sex.

The animals were lightly anesthetized with ether, and the hair was clipped from the sides and back. The substance was applied to the center of the back and spread over an area of 25-50 cm^2. An attempt was made to cover the same area on each animal and to apply the substance uniformly. A microliter pipetting device was used to apply 2 ml/kg body weight of undiluted sample. The rats were then placed in individual cages where they remained for 4 hr. During this time they were observed for signs of systemic toxicity. At the end of the 4 hr period any residual substance was removed and signs of skin injury were noted. The animals were returned to maintenance cages and observed over a period of 14 days. At the end of this period, body weights were obtained and necropsies were performed.

Delayed-Type Allergic Contact Hypersensitivity in Guinea Pigs

The objective was to determine the delayed-type allergic response resulting from cutaneous contact with a substance. The animals used for this test were Hartley albino male and female guinea pigs about 3 months old. Each substance was tested on four different animals of each sex.

Hair was clipped from a 3- to 4-cm^2 area on the back between the shoulder blades. Clipping was done on the first day of injection and when necessary during the course of further injections. A 0.1-ml portion of a 0.1% solution of the substance in 20% DMSO-80% saline or in propylene glycol was injected intradermally three times a week for 3 weeks. The injections were within a 3- to 4-cm^2 field at different sites within the field. Twelve days after the ninth injection, a challenge dose of 0.05 ml of the 0.1% solution of the substance was injected intradermally into a fresh site of the clipped area. Readings for skin reactions were made 24 and 48 hr after each of the nine injections and after the challenge dose. The response was graded and evaluated according to the method of Draize et al. (2) which scores sensitization in terms of redness, edema, blistering, and necrosis.

Primary Eye Irritation in Rabbits

The objective was to determine surface irritation to ocular tissues by a substance following a single application. The animals used were New Zealand albino male rabbits that weighed about 2 kg. Each substance was tested on one eye of four animals; the other eye served as a control. The lower lid was gently pulled away from the eye, and 0.1 ml of the undiluted agent was instilled into the conjunctival sac by use of a pipetting device. The eyes were examined for signs of irritation 1, 2, and 3 days after instillation. The response was graded and evaluated according to a modified Draize method (2) which scores irritation in terms of redness of the lid and the conjunctiva, edema of the lid and conjunctiva, and opacity of the eye surface.

Primary Skin Irritation in Rabbits

The objective was to determine skin irritation by a substance following a single application. The animals used for this test were New Zealand albino male rabbits that weighed about 2 kg. Each substance was tested on six different animals.

Hair was clipped from the sides of the rabbits, and 0.5 ml of the undiluted substance was distributed evenly over a 6- to 7-cm^2 area of flank skin by use of a pipetting device. The area was covered with a gauze pad secured with strips of adhesive tape, and the flank was wrapped with an elastic bandage. Four hours later, the bandage and pad were removed and any residual substance was wiped off with dry gauze. At this time the first reading for a skin response was made, followed by further readings at 24 and 72 hr after application. If a definite response was observed at 72 hr, another reading was made 7 days after application. The response was graded and evaluated according to the method of Draize et al. (2) which scores irritation in terms of redness and edema of the skin.

Histopathology in Mice

Male BALB/c mice 6-8 weeks old, 10 per group, were given a single ip injection of the test agent. The mice were killed 2 or 4 days later, and gross necropsy was performed. Tissues were removed and fixed in 10% buffered formalin. They were embedded in paraffin, sectioned at 4-6 μm, and stained with hematoxylin and eosin. The following organs were examined histologically: brain, heart, liver, kidney, esophagus, stomach, intestine, colon, pancreas, spleen, gall bladder, adrenal, lung, trachea, lymph node, and thymus.

Lung Adenoma Assay in Strain A Mice

The objective was to determine if multiple ip injections of a substance can induce lung adenomas in strain A/Jax male mice 6-8 weeks old. In preliminary toxicity studies, the maximum tolerated dose (MTD) was determined. The MTD is defined as the highest dose producing no mortality when given in a total of six injections over a 2-week period. After 6 weeks, survivors were killed and gross necropsies were performed. In the adenoma test proper, each substance was injected into 30 mice three times a week (Monday, Wednesday, Friday) for 8 weeks. The following doses were used: the MTD, one-half the MTD, and one-fifth the MTD. Control groups were a) 30 mice injected with the same vehicle (corn oil) used to dissolve the test substance, b) 50 mice given a single injection of urethan at 1 g/kg body weight (positive control), and c) 50 mice which received nothing.

Body weights were recorded every 2 weeks, and the mice were killed 20 weeks after the first injection. The lungs were fixed in Tellyesniczky's solution and the number of tumors on the lung surface was counted. Selected tissues were fixed in 10% buffered formalin and Bouin's fixatives for histopathology.

RESULTS

Acute Toxicity in Mice and Rats

Data for acute toxicity of shale oil and its derivatives are given in Table I. Crude Shale Oil was the only one that produced acute deaths in both male (at doses of 8 and 10 g/kg) and female (at doses of 4, 8, and 16 g/kg) mice when given orally. Following ip injection, deaths also occurred at doses of 2 g/kg. Because the mortality data were similar for both sexes, substances 4608-4610 were tested in female mice only. The LD_{50} was between 6 and 8 g/kg for substances 4608 and 4609 and greater than 16 g/kg for substance 4610.

Results for petroleums and derivatives are shown in Table II. Acute toxicity data were for all practical purposes similar to the ones observed with shale oil and its derivatives.

In both male and female rats, there were no visible toxic responses arising from the dermal application of 2 g/kg of samples 4601, 4602, and 4607. Neither visible skin lesions nor manifestations of central nervous system perturbations were observed. Deaths did not occur during the 2 weeks

Table I

Acute Lethality Test in Mice: Shale Oil and Its Derivatives

	LD_{50} (g/kg)			
	Oral		Intraperitoneal	
Substance	Male	Female	Male	Female
4601	11.3	11.3	6.1 (4.5-8.2)[a]	4.3 (2.0-6.1)
4602	>16.0	>16.0	>16.0	>16.0
4607	>16.0	>16.0	>16.0	>16.0
4608	ND[b]	>16.0	ND	8.0 (5.4-12.8)
4609	ND	>16.0	ND	6.4 (5.0-8.0)
4610	ND	>16.0	ND	>16.0

[a]95% confidence limits.

[b]ND = not done.

Table II

Acute Lethality Test in Mice: Petroleums and Their Derivatives

Substance	LD_{50} (g/kg) Oral Male	Oral Female	Intraperitoneal Male	Intraperitoneal Female
5107	>10.0	ND[a]	ND	ND
5301	>16.0	ND	ND	ND
5305	>16.0	ND	ND	ND
4614	ND	>16.0	ND	11.2 (8.13-15.5)[b]
4615	ND	>16.0	ND	8.0 (5.5-12.8)
4616	ND	>16.0	ND	>16.0

[a]BD = not done.

[b]95% confidence limits.

following application of the substances, and all rats showed a progressive gain in body weight (Table III). Similar observations were made when rats were treated with 2 g/kg of Crude Shale Oil- or petroleum-derived JP-5, JP-8, or Diesel Fuel Marine product: no animals died, no skin lesions developed, and weight gains over a 2-week period were normal.

Delayed-Type Contact Hypersensitivity

Guinea pigs were sensitized with Crude Shale Oil, Hydrotreated Shale Oil, Hydrotreated Residue, or shale oil- and petroleum-derived jet and diesel fuels. The data are summarized in Table IV. During the sensitization procedure, only slight redness and focal scab formation were noted in some of the animals for all nine substances; edema and necrosis were not seen. After the challenge injection, all animals sensitized with Shale Oil, Hydrotreated shale oil, and Hydrotreated Residue developed a degree of redness and edema greater than that observed after the sensitizing injections. The challenge injection of these three substances also produced a slight necrosis in most guinea pigs, and for two of the substances, a marked lesion with a necrotic center developed. No difference in reaction between the sensitizing and challenge injection was observed in animals exposed to the shale oil- or petroleum-derived jet and diesel fuels.

Eye and Skin Irritation in Rabbits

The data in Table V show that only the shale oil and to a lesser extent its hydrotreated derivative produced a visible redness of the rabbit eye. However, irritation was slight and transient and is considered insignificant in evaluating substance toxicity. None of the other substances produced visible irritation at the three observation intervals. There were no indications that the shale oil, its five derivatives, or the petroleum products were irritating to rabbit skin to the extent of producing visible redness or edema (Table VI).

Histopathology

Mice killed 2 or 4 days following ip injection of 4601 at 4 g/kg, or 4602 or 4607 at 16 g/kg showed superficial pyogranulomatous serositis of the peritoneal cavity resulting from local reaction to the substances. Lymphoid depletion of the thymic cortex was present in all animals at 2 and 4 days, but was most marked in mice injected with 4602. Serositis and thymic cortical depletion were the only histopathologic alterations observed for these

Table III

Weight Gain in Male and Female Rats Following Dermal Application of 2 g/kg of Shale Oil, Hydrotreated Shale Oil, or Hydrotreated Residue[a]

	Mean Body Weight (g) ± SE			
	Male		Female	
Substance	Day 0	Day 14	Day 0	Day 14
4601	358 ± 8	415 ± 7	209 ± 5	241 ± 2
4602	352 ± 9	422 ± 9	209 ± 3	238 ± 4
4607	370 ± 4	435 ± 7	225 ± 5	256 ± 6

[a]Six animals per group except for substance 4602 females, for which there were 5 rats.

substances. No lesions were present in animals injected with corn oil alone.

Lung Adenoma Assay

This assay was done essentially as described by Shimkin and Stoner (3). In preliminary toxicity studies, the MTD for 4601 was determined to be 2.5 g/kg per injection and for 4602, 16 g/kg. However, during the course of the adenoma assay it was found that the MTD, as determined, was too high. Only in one group was the survival rate higher than 50%. Cumulative mortality rates are shown in Figure 1A and B, and the average weight gain of survivors is shown in Figure 2. It is interesting to note that with both Crude Shale Oil and Hydrotreated Shale Oil animals continued to die even after cessation of exposure to the test substances. The reasons for this delayed toxicity remain to be established. Treated animals also generally failed to gain weight at rates comparable to that of controls.

As shown in data of Table VII, tumor incidence was 30-60% in treated animals and not significantly different from animals injected with vehicle (corn oil) alone. On the other hand, tumor multiplicity was significantly higher in animals injected with 1.25 g/kg of Crude Shale Oil. In all other groups, the differences from the control value were statistically not significant. However, few animals survived when injected with 2.5 g/kg of Crude Shale Oil or with 16, 8, or 3.2 g/kg of Hydrotreated Shale Oil. In view of

Table IV

Delayed-Type Allergic Contact Hypersensitivity Test in Guinea Pigs

Substance	Average Response[a] 24 and 48 Hr After Each of Nine Sensitizing Injections			Average Response[a] 24 and 48 Hr After the Challenge Injection		
	Redness	Edema	Necrosis	Redness	Edema	Necrosis
Shale Oil and Its Derivatives						
4601	±	0	0	1-2	2	±
4602	0	0	0	2	1	±
4607	0	0	0	2	1	±
4608	0-1	0	0	0	0	0
4609	0-1	0	0	0	0	0
4610	0-1	0	0	0	0	0
Petroleum Derivatives						
4614	0-1	0	0	0	0	0
4615	0-1	0	0	0	0	0
4616	0-1	0	0	0	0	0
Propylene Glycol[b]	0-1	0	0	0	0	0
Dinitrochlorobenzene[c]	1-2	0-1	1-2	1-2	1-2	2-3

[a]Scale: 0, ±, 1-4.

[b]Vehicle.

[c]Positive control.

Table V

Primary Eye Irritation Test in Rabbits

Substance	Response at Indicated Times after Instillation[a]		
	24 Hr	48 Hr	72 Hr
Shale Oil and Its Derivatives			
4601	6 of 6 showed grade 1 redness[b]	0	0
4602	2 of 6 showed grade 1 redness[b]	0	0
4607	0	0	0
4608	0	0	0
4609	0	0	0
4610	0	0	0
Petroleum Derivatives			
4614	0	0	0
4615	0	0	0
4616	0	0	0

[a]Four or six eyes per substance.

[b]On the basis of 0-4 response of lid, conjunctiva, and cornea.

Table VI

Primary Skin Irritation Test in Rabbits

Substance	No. of Rabbits Responding at Indicated Time after Application[a]					
	4 Hr		24 Hr		72 Hr	
	Redness	Edema	Redness	Edema	Redness	Edema
Shale Oil and Its Derivatives						
4601	0	0	0	0	0	0
4602	0	0	0	0	0	0
4607	0	0	0	0	0	0
4608	0	0	0	0	0	0
4609	0	0	0	0	0	0
4610	0	0	0	0	0	0
Petroleum Derivatives						
4614	0	0	0	0	0	0
4615	0	0	0	0	0	0
4616	0	0	0	0	0	0

[a]Six animals per substance.

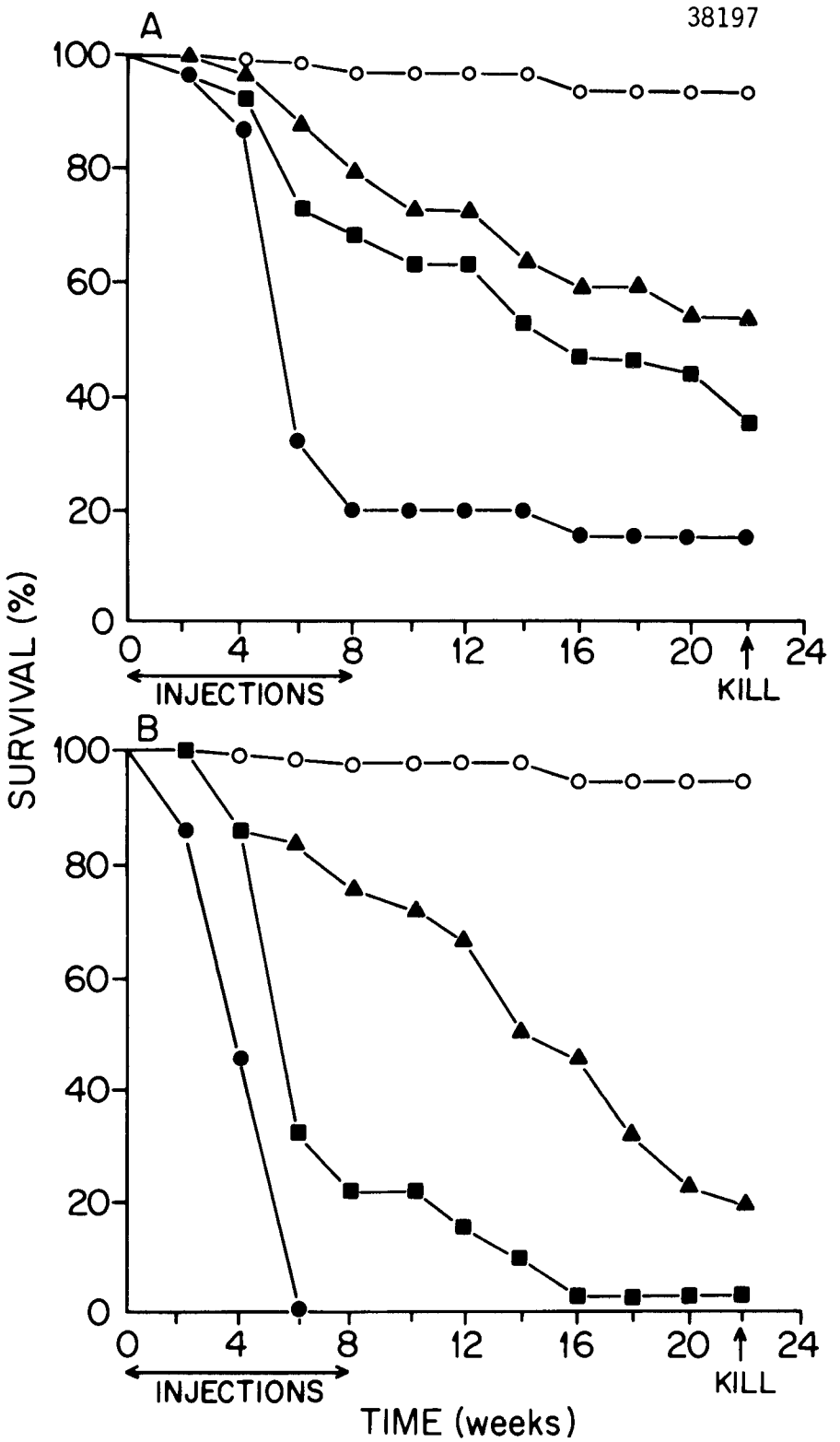

Figure 1. Survival of mice given (A) 24 injections of crude shale oil 4601 or (B) hydrotreated shale oil 4602. (A) ●, 2.5 g/kg; ■, 1.25 g/kg; ▲, 0.5 g/kg; o, corn oil. (B) ●, 16 g/kg; ■, 8 g/kg; ▲, 3.2 g/kg; o, corn oil. Brackets indicate SE's.

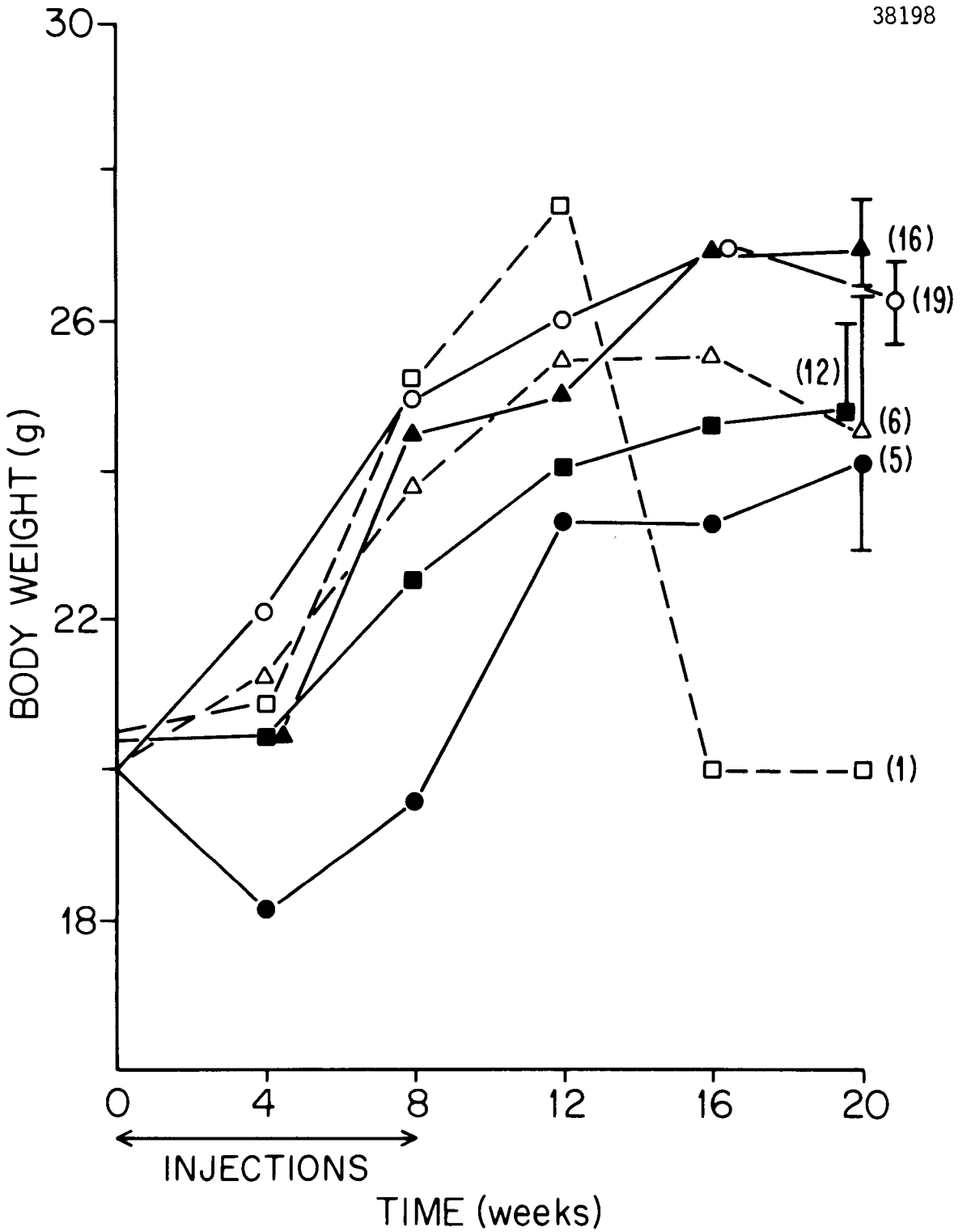

Figure 2. Body weight of mice given 24 injectsions of crude shale oil 4601 or hydrotreated shale oil 4602. 4601: ●, 2.5 g/kg; ■, 1.25 g/kg; ▲, 0.5 g/kg. 4602: □, 8 g/kg; △, 3.2 g/kg; ○, corn oil. Number of mice shown in parentheses. Brackets indicate SE's.

Table VII

Mouse Lung Adenoma Assay: Shale Oil and Hydrotreated Shale Oil

Substance	Dose per Injection[a] (g/kg)	No. of Mice Initially	No. of Survivors	No. of Mice with Tumors	No. of Tumors/Mouse[b]
4601	2.5	30	5	3	0.6 ± 0.3
	1.25	30	12	6	1.3 ± 0.5
	0.5	30	16	7	0.6 ± 0.2
	0.25	30	18	3	0.2 ± 0.1
4602	16	30	0	-	-
	8	30	1	0	0
	3	30	6	3	1.0 ± 0.6
	1.6	30	19	4	0.4 ± 0.2
	0.6	30	21	3	0.1 ± 0.1
4607	8	30	6	6	6.17 ± 1.20
	4	30	8	6	1.75 ± 0.63
	1.6	30	6	3	0.67 ± 0.36
Corn Oil	10	20	19	6	0.4 ± 0.1
Untreated	-	20	20	3	0.2 ± 0.1
Urethan[c]	1.0	20	20	20	23.2 ± 1.7[d]

[a]Given three times a week for 8 weeks.

[b]Total number of tumors divided by total member of surviving animals; mean ± SE.

[c]One ip injection of 1 mg/g body weight.

[d]$p < 0.05$ compared with corn oil control.

this general toxicity, the adenoma assay in these groups is probably not representative and needs to be repeated at lower dose levels. Detailed results on histopathology will be reported later.

DISCUSSION

Our data show that, when ingested, Crude Shale Oil may be considered slightly toxic and all other samples are practically nontoxic; no sex difference was found where both male and female mice were tested. Following ip injection the substances were slightly toxic. Acute skin toxicity was invariably greater than 2 g/kg, and no evidence was obtained to document appreciable eye and skin irritation. Histopathologic findings following acute administration of large amounts of test material were not remarkable. Lymphoid reduction in the thymic cortex was interpreted as a stress reaction rather than a substance-specific effect.

Compared with petroleum and some of its derivatives, shale oil and its derivatives appear to have practically the same overall low toxicity. Fuels from both sources have about the same LD_{50}'s and are essentially nonirritating to the skin and eye. These observations corroborate our earlier studies which showed that three other petroleum-based substances did not elicit skin or eye toxicity (Table VIII). We would not expect, therefore, that acute exposure to these jet and diesel fuels produced from shale oil would be any more hazardous than exposure to their petroleum-derived equivalents currently in use.

From the present data, we conclude that a single exposure of humans to any one of the six Paraho/Sohio substances tested would result in little if any systemic toxicity or skin or eye injury. One possible exception arises from the findings of the skin-sensitization bioassay. Our data suggest that repeated topical exposures to the Crude Shale Oil or the hydrotreated derivatives 4602 or 4607 could possibly elicit an immunologically mediated reaction in humans. This type of reaction which could take weeks or months to develop may become manifest as itching, reddening, eruption, and edema of the skin. These reactions may disappear if there is no further contact with the sensitizing substance, but the state of sensitization may be permanent.

The data obtained in the lung adenoma bioassay suggest that Crude Shale Oil has tumorigenic potential. This appears to agree with preliminary data obtained in skin painting experiments (6). Before any more definitive

Table VIII

Skin and Eye Toxicity of Petroleum-Based Substances

Substance	Chemical Repository No.	Acute LD_{50}, Skin (g/kg)	Eye Irritation	Skin Irritation
Syncrude	1106	>2	0	0
Mixed Petroleum Crude	5107	>2	0	0
Synthoil Crude	1201	>2	0	0

conclusions can be drawn, particularly as to the carcinogenic potency of shale oil, additional studies with the lung adenoma bioassay will have to be completed.

It is interesting to note that the oral LD_{50}'s of shale oil, petroleum, and their derivatives are consistently higher than the oral LD_{50} found with materials derived from various coal conversion processes. Table IX lists some data for comparative purposes.

In conclusion, acute toxicity of Paraho Crude Shale Oil and of five derivatives does not appear to be a problem of immediate concern. The substances produced slight acute effects similar to those in a report by Weaver and Gibson (4), except that they failed to obtain a positive response in the assay for skin sensitization. This discrepancy might need to be resolved in further studies.

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Table IX

Acute Oral Lethality Test of Selected Coal Conversion Products in BALB/c Male Mice

Substance	Chemical Repository No.	LD_{50} (g/kg)
H-Coal (PDU samples)		
H-Coal Distillate (Raw)	1601	3.6 (2.8-4.5)[a]
HDT Distillate (Low Severity)	1602	4.0 (3.4-4.7)
HDT Distillate (High Severity)	1604	5.5 (3.8-7.2)
HDT Fuel Oil (Low Severity)	1617	6.2 (3.7-8.7)
HDT Fuel Oil (High Severity)	1619	6.2 (5.1-7.3)
Atmospheric Still Bottoms (ASB) (Fuel Oil Mode)	1313	2.3 (1.9-2.6)
Vacuum Still Overhead (VSOH) (Fuel Oil Mode)	1314	2.6 (2.2-3.2)
Atmospheric Still Bottoms (ASB) (Syncrude Mode)	1309	3.6 (2.4-5.2)
Vacuum Still Overhead (VSOH) (Syncrude Mode)	1310	2.5 (1.7-3.1)
Coal SRC I		
HDT Light Organic Liquid (Low Severity)	1606	3.6 (3.1-4.1)
HDT Light Organic Liquid (High Severity)	1608	10.0
HDT Recycle Solvent (Low Severity)	1614	2.6 (1.9-3.2)
HDT Recycle Solvent (High Severity)	1616	5.3 (4.4-6.3)
Coal SRC II		
Fuel Oil Blend	1701	3.1 (2.7-3.5)
Zinc Halide Hydrocracking Process		
Product Distillate	1801	3.4 (0.5-2.3)

[a]95% confidence limits.

SECTION 3: THE CHRONIC DERMAL TOXICITY OF PARAHO SHALE OIL AND SHALE OIL DISTILLATES

INTRODUCTION

Experiments were conducted to determine the chronic dermal toxicity of an above ground retort shale oil produced by the Paraho process. Samples were obtained through the ORNL repository and represent materials produced under the auspices of the U.S. Navy to evaluate the suitability of shale oil as an alternative to petroleum. To place the shale data in perspective, natural petroleum derived distillates, comparable in specification to the shale oil materials, were also tested.

MATERIALS AND METHODS

Animal Exposures

At ten weeks of age male and female C3Hf/Bd mice were randomly distributed to test and control groups. Animals were housed 5 per standard cage on sterile hardwood chip bedding and given Purina 5010C and hyperchlorinated-acidified water, ad. lib.

For the purpose of examining the effect of dose rate on cumulative risk for further skin tumor induction or systemic toxicity, two parallel experiments were run. Materials were applied either for 40 weeks at three times per week or twice weekly for 60 weeks. Animals treated for 40 weeks were kept under observation until after the 60-week exposures were complete. Approximately the same total quantity of material was administered to both groups.

Test Materials

Details concerning the source, identity and chemical characteristics of the materials used in this study can be found elsewhere (1,2). Density was determined by applying 0.02 ml volumes of each material to preweighed circles of filter paper. The filter papers were immediately reweighed and the net weight per unit volume calculated. The densities determined by this procedure may be slightly different than those derived by other means; however, the method has the advantage that it closely approximates the actual materials handling and application conditions used in the cutaneous carcinogenesis bioassay. Therefore the data are relevant to the amounts of material applied to mouse skin. The solvent for the samples was cyclohexane and the volume per application was 50 μl.

RESULTS

Skin Tumor Induction

The skin tumorigenicity of the raw shale oil, the hydrotreated shale, and the hydrotreated residue is indicated in Table I. It is apparent from these data that Paraho shale oil is carcinogenic in mouse skin, that skin carcinogenicity is reduced but not eliminated by hydrotreatment and that the hydrotreated residue still retains appreciable activity. A more precise evaluation of the carcinogenic potency, relative to benzo(a)pyrene, is in progress.

Comparison of the 3/wk and 2/wk data, at comparable total dose, reveals that both dose rates are equally effective in inducing skin tumors, for both the crude shale oil as well as the hydrotreated residue. However, the response obtained with the hydrotreated shale oil was reduced by protraction of the exposure.

Distillates derived from both shale oil and natural petroleum exhibited weak skin carcinogenic activity. Again, more precise evaluation of these data, based not only upon frequency but also the time to appearance of the tumor, is in progress. Table II summarizes the observed skin tumor frequencies and latency in mice exposed to shale oil distillates and Table III, the data for comparable natural petroleum derived materials. The shale derived JP-5 appeared slightly more active than JP-8 which in turn was more active than DFM. Comparing the shale and petroleum derived distillates on the basis of overall frequency as well as observed latencies suggested that the shale based materials were slightly more active. More extensive analysis will be necessary, however, to determine if these differences are statistically significant. The petroleum JP-5 also appeared slightly more active than either JP-8 or DFM, which were comparable.

Systemic Toxicity

Paraho shale oil and distillates were also evaluated for systemic toxicity. Mortality, body and organ weights were determined as well as clinical evaluation of kidney function. The mortality, body and organ weight data are summarized in Table IVA for female and IVB for male mice at the 3 times per week dose rate.

Significant reduction in body weight was noted in both male and female mice with the female appearing to be more sensitive on the basis of degree

Table I

Skin Tumor Frequencies and Average Latency in C3H Mice
Exposed to Paraho Process Shale Oil Before and After Upgrading

Material (ORNL No.)	Density (gm/cc)	Dose Per Application (mg)	Applications Per Week	No. Tumor Positive[a] Female (%)	Male (%)	Average Latency[b] Female	Male
Crude Shale Oil	0.990	24.8	3	13(87)	15(100)	222(6)	198(9)
(4601)		24.8	2	10(100)	10(100)	266(11)	299(21)
		12.4	2	10(100)	9(90)	369(31)	332(17)
		6.2	2	4(40)	6(60)	373(91)	498(32)
Hydrotreated	0.925	24	3	5(33)	10(67)	254(21)	222(14)
Shale Oil		24	2	1(10)	4(40)	420	362(30)
(4602)		12	2	0	0	--	--
		6	2	0	0	--	--
Hydrotreated	0.865	23.2	3	8(53)	15(100)	246(18)	187(17)
Residue		23.2	2	8(80)	8(80)	406(24)	326(28)
(4607)		11.6	2	0	3(30)	--	557(47)
		5.8	2	1(10)	0	569	--
Cyclohexane	--	0.050	3	0	0	--	--
Benzo(a)pyrene		0.050	3	15(100)	15(100)	173(6)	164(11)
		0.025	3	15(100)	15(100)	209(6)	220(4)
		0.0125	3	15(100)	15(100)	262(7)	274(7)

[a] 15 per dose, each sex, at 3/wk. 10 per dose, each sex at 2/wk. 30, each sex, cyclohexane control.
[b] Days ± standard error.

Table II

Skin Tumor Frequency and Average Latency in C3H Mice Exposed to Shale Oil Derived Commercial Distillates

Material (ORNL No.)	Density (gm/cc)	Dose Per Application (mg)	Applications Per Week	No. Tumor Positive[a] Female (%)	Male (%)	Average Latency[b] Female	Male
JP-5	0.845	42.2	3	3(20)	1(7)	389(19)	480
4608		22.9	3	4(27)	3(20)	453(19)	484
		42.2	2	1(10)	3(30)	417	425(2)
		22.9	2	1(10)	1(10)	434	441
		11.4	2	2(20)	1(10)	623(7)	484
		5.7	2	2(20)	0	486(32)	--
JP-8	0.830	41.5	3	2(13)	3(20)	466(12)	394(49)
4609		22.7	3	2(13)	1(7)	414(34)	480
		41.5	2	0	0	--	--
		22.7	2	0	0	--	--
		11.3	2	4(40)	1(10)	556(54)	652
		5.7	2	0	1(10)	--	665
DFM	0.900	45	3	2(13)	0	478	--
4610		23.7	3	0	1(7)	--	478
		45	2	0	0	--	--
		23.7	2	0	0	--	--
		11.8	2	0	0	--	--
		5.9	2	0	0	--	--

[a]15 per dose, each sex, at 3/wk. 10 per dose, each sex at 2/wk. 30, each sex, cyclohexane control.
[b]Days ± standard error.

Table III

Skin Tumor Frequency and Average Latency in C3H Mice Exposed to Petroleum Derived Commercial Distillates

Material (ORNL No.)	Density (gm/cc)	Dose Per Application (mg)	Applications Per Week	No. Tumor Positive[a] Female (%)	Male (%)	Average Latency[b] Female	Male
JP-5	0.845	42.2	3	0	0	--	--
(4614)		22.9	3	1(7)	0	434	--
		42.2	2	1(10)	1(10)	371	456
		22.9	2	1(10)	0	428	--
		11.4	2	4(40)	0	620(35)	--
		5.7	2	0	1(10)	--	669
JP-8	0.835	41.8	3	0	2(13)	--	452(32)
(4615)		22.8	3	1(7)	0	420	--
		41.8	2	0	0	--	--
		22.8	2	0	0	--	--
		11.4	2	0	0	--	--
		5.7	2	1(10)	0	651	--
DFM	0.91	45.5	3	1(7)	1(7)	420	479
(4616)		23.8	3	0	1(7)	--	485
		45.5	2	0	0	--	--
		23.8	2	1(10)	0	413	--
		11.9	2	0	1(10)	--	673
		6.0	2	0	0	--	--

[a] 15 per dose, each sex, at 3/wk. 10 per dose, each sex at 2/wk. 30, each sex, cyclohexane control.
[b] Days ± standard error.

Table IV-A

Mortality and Weight Comparisons for Shale Oil, Shale Oil Distillate and Petroleum Distillate in Female C3H Mice

Material	Dosage (mg/wk)	No. of Deaths (%)	Average Weight at Necropsy (SE)[a]				
			Body (gm)	Liver (gm)	Spleen (mg)	R. Kidney (mg)	L. Kidney (mg)
4601	74.4	10(67)	25(1.0)	--	--	--	--
4602	72	2(13)	28(1.2)	--	--	--	--
4607	69.6	8(53)	25(1.7)	--	--	--	--
4608	126.6	1(7)	23(1.1)	1.589(0.1)	114(5)	271(14)	235(17)
	68.7	1(7)	25(0.8)	1.784(0.2)	140(14)	284	--
4609	124.5	4(27)	26(0.9)	1.891(0.2)	164(72)	285(20)	257(28)
	62.2	5(33)	25(0.7)	1.726(0.7)	149(17)	275(15)	266(14)
4610	135	4(27)	23(1.8)	2.109(0.1)	187(23)	278(15)	237(15)
	67.5	1(7)	26(0.7)	2.062(0.1)	213(17)	310(12)	291(9)
4614	126.6	6(40)	25(0.5)	1.777(0.1)	123(14)	303(20)	241(20)
	63.3	1(7)	26(0.7)	1.757(0.7)	141(12)	274(10)	269(9)
4615	125.4	4(27)	24(1.1)	1.717(0.8)	97(7)	255(15)	223(12)
	62.7	0	26(0.5)	1.636(0.5)	136(13)	275(9)	246(9)
4616	136.5	6(40)	22(1.2)	1.651(0.8)	143(26)	277(20)	244(16)
	68.2	2(13)	27(0.9)	1.813(0.5)	149(14)	295(19)	271(9)
Cyclohexane	--	2(13)	28(0.6)	1.660(0.4)	125(9)	262(7)	249(10)

[a]Values underlined were significantly different than vehicle control ($P < 0.05$, students-T).

Table IV-B

Mortality and Weight Comparisons for Shale Oil, Shale Oil Distillate and Petroleum Distillate in Male C3H Mice

Material	Dosage (mg/wk)	No. of Deaths (%)	Average Weight at Necropsy (SE)[a]				
			Body (gm)	Liver (gm)	Spleen (mg)	R. Kidney (mg)	L. Kidney (mg)
4601	74.4	11(73)	27(1.5)	--	--	--	--
4602	72	5(33)	30(0.9)	--	--	--	--
4607	69.6	9(60)	27(1.5)	--	--	--	--
4608	126.6	1(7)	27(0.8)	1.735(0.9)	111(9)	337(19)	313(23)
	68.5	0	28(0.6)	1.616(0.4)	113(11)	308(9)	304(8)
4609	124.5	2(13)	26(0.7)	1.433(0.1)	135(24)	325(13)	306(18)
	62.2	0	31(0.5)	1.725(0.3)	115(9)	347(11)	332(12)
4610	135	3(20)	26(0.9)	1.711(1.0)	144(16)	355(9)	315(12)
	67.5	1(7)	28(0.7)	1.8(0.1)	182(21)	315(9)	302(8)
4614	126.6	2(13)	27(0.6)	1.793(0.8)	148(21)	330(13)	327(12)
	63.3	0	301(0.6)	1.798(0.2)	136(17)	344(11)	321(11)
4615	125.4	1(7)	27(0.6)	1.731(0.6)	126(16)	324(7)	311(8)
	62.7	0	29(0.6)	1.744(0.4)	106(8)	363(10)	319(18)
4616	136.5	3(20)	26(1.2)	1.833(0.1)	158(17)	346(13)	343(14)
	68.7	1(7)	27(0.6)	1.790(0.8)	168(15)	320(7)	300(10)
Cyclohexane	--	2(13)	30(0.6)	1.592(0.4)	99(6)	417(12)	407(10)

[a]Values underlined were significantly different than vehicle control ($P < 0.05$, students-T).

and consistency of effect. Liver weight was unaffected in either sex. Spleen weight was significantly elevated. This represents a non-specific response to the inflammatory skin lesions induced by these materials as well as inflammatory complications arising from skin neoplasms. Kidney weights were consistently reduced in male mice exposed to the distillates. In females this effect was more variable. The reduction and variability of renal weight may be interpreted in light of clinical and histopathological evidence of kidney damage that appeared to be treatment-, dose- and sex-dependent. An increased mortality, relative to control, was especially obvious in groups experiencing high incidences of malignant skin cancer. More sporadic but still slightly elevated frequencies were seen among the distillate groups.

Renal Toxicity

A dose dependent increase in water intake was noted in groups exposed to the distillate materials but not the crude shale oil, the hydrotreated shale oil or hydrotreated residue (Table V). It was possible that this effect was due, at least in part, to renal toxicity. To test this hypothesis, treated animals were placed in metabolism cages which permitted accurate measurement of water intake and urine production. The urine collected was further analyzed to evaluate renal concentrating ability. Representative data for both the shale and petroleum base DFM and also vehicle control is given in Table VI. A statistically significant increase in urine output accompanied by a reduction in osmolarity was noted at the highest distillate dose levels. Renal lesions were noted at terminal sacrifice, with a frequency that paralleled the elevation in water consumption.

Affected kidneys were atrophic with a pale nodular surface. Microscopically, the cortex exhibited focal and segmental tubular atrophy that varied in extent and severity. In extreme cases, when lesions were both severe and bilateral, kidney damage was sufficient to have been the cause of death. Very little fibrosis was noted and, while amyloid deposition, especially in the hepatic sinusoids, was noted in many individuals with chronic renal pathology, amyloid deposition in the kidney was insufficient to account for either the severity or distribution of the lesions, and many animals with advanced renal disease had no evidence of amyloidosis. In addition to the cortical lesions, papillary necrosis was also observed; nevertheless, as was the case with amyloidosis, papillary necrosis was an

Table V

Average Daily Water Consumption in Mice Exposed to Shale Oils and Distillates for a Minimum of 30 Weeks

	3/Week Exposures		2/Week Exposures			
	Concentrations:		Dose Per Application:			
Materials	100	50%(w/v)	100	50	25	12%(w/v)
4601	nt[a]	nt	--	5.1(0.3)	4.9(0.1)	5.1(0.1)
4602	nt	nt	--	5.0(0.1)	4.7(0.2)	4.7(0.2)
4607	nt	nt	--	4.8(0.3)	4.0(0.1)	4.8(0.3)
4608	9.9(0.3)[b,c]	7.3(0.3)	8.3(0.3)	6.6(0.1)	5.7(0.3)	4.7(0.1)
4609	9.4(0.4)	7.3(0.3)	8.2(0.2)	6.7(0.3)	5.7(0.3)	5.1(0.2)
4610	10.9(0.3)	7.8(0.2)	9.2(0.4)	6.9(0.2)	6.1(0.3)	5.0(0.1)
4614	10.4(0.4)	7.3(0.2)	8.1(0.1)	6.7(0.3)	5.1(0.1)	4.4(0.1)
4615	10.2(0.3)	7.5(0.1)	8.2(0.1)	6.8(0.1)	5.4(0.3)	4.9(0.2)
4616	10.2(0.3)	7.7(0.2)	9.3(0.2)	6.8(0.2)	5.4(0.1)	5.3
Cyclohexane	5.1(0.1)	--	--	--	--	--

[a]Not tested.

[b]ml/mouse/day.

[c]Values underlined are significantly different than cyclohexane control ($P < 0.05$).

Table VI

Urine Characteristics in Mice Undergoing Chronic Dermal Exposure to Middle Distillate

Material	Sex	Concentration (w/v %)	Samples	Urine Output (ml/Mouse/Day)	Osmolarity (MOsmoles)	pH	Protein (mg/ml)
Cyclohexane	F	100	6	1.0(0.1)	398(128)	6.1(0.4)	0.8(0.3)
Shale Oil DFM	F	100	4	3.4(1.1)[a]	121(20)	6.0(0.6)	0.5(0.1)
Shale Oil DFM	F	50	4	1.3(0.2)	217(66)	6.0(0.04)	0.7(0.2)
Petrol. DFM	F	100	3	2.1(0.8)	119(17)	5.8(0.09)	0.5(0.1)
Petrol. DFM	F	50	5	1.2(0.2)	298(45)	5.9(0.9)	0.7(0.1)
Cyclohexane	M	100	11	1.0(0.1)	398(63)	6.2(0.06)	2.1(0.4)
Shale Oil DFM	M	100	5	1.2(0.3)	383(54)	6.0(0.04)	1.3(0.1)
Shale Oil DFM	M	50	5	1.7(0.3)	287(39)	6.0(0.07)	1.4(0.3)
Petrol. DFM	M	100	3	1.6(0.08)	274(40)	6.0(0.07)	1.0(0.3)
Petrol. DFM	M	50	6	1.6(0.3)	380(46)	6.0(0.09)	1.5(0.1)

[a]Values underlined are significantly different than control at the 0.05 level.

inconsistent observation and thus was not considered to be causally related to the cortical changes.

At the time of this report efforts are underway to reproduce the renal damage, acutely, by direct parenteral administration of shale and petroleum based DFM. If we are successful it will facilitate mechanistic studies without which it will be impossible to relate these findings to human health.

SUMMARY AND CONCLUSIONS

Paraho shale oil is carcinogenic in mouse skin. Mild hydrotreatment reduces but does not eliminate skin carcinogenicity and appreciable carcinogenic activity remains in the residue material. Protraction of the exposure importantly influences the response but not in a predictable manner. For some materials a diminished effect was noted at reduced dose rate; however, for others the overall risk remains either unchanged or appeared to increase. These data are consistent with the view that, for each carcinogenically active material, there is an optimum dose as well as dose rate.

Kidney injury was noted following chronic dermal exposure to shale and petroleum derived middle distillates. The importance of this observation to human health is uncertain; however, there have been reports of acute renal failure following human exposure to diesel fuel (3,4). It must be emphasized that the nephrotoxic effect is not specific to shale oil but is common to both petroleum and shale oil derived middle distillates.

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