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NTIS - 25

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EFFECTS OF AQUEOUS EFFLUENTS FROM IN SITU FOSSIL FUEL PROCESSING
TECHNOLOGIES ON AQUATIC SYSTEMS

By
Harold L. Bergman

January 1980

Work Performed Under Contract No. DE-AS20-79-LC-01761

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Laramie, WY 82071

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UNITED STATES DEPARTMENT OF ENERGY

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ANNUAL PROGRESS REPORT

1 January - 31 December 1979

EFFECTS OF AQUEOUS EFFLUENTS FROM IN SITU FOSSIL
FUEL PROCESSING TECHNOLOGIES ON AQUATIC SYSTEMS

Edited by

Michael D. Marcus and Harold L. Bergman

Submitted to

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under
Contract #DE-AS20-79-LC-01761; Task Agreement #001
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The Rocky Mountain Institute of Energy and Environment
The University of Wyoming

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4 January 1980

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1.0 SUMMARY

This is the third annual progress report for a continuing EPA-DOE jointly funded project to evaluate the effects of aqueous effluents from in situ fossil-fuel processing technologies on aquatic biota. The project is organized into four project tasks: (1) literature review, (2) process water screening, (3) methods development and (4) recommendations. Accomplishments in the four project tasks during 1979 are summarized as follows:

Task 1: Literature Review

- Our major accomplishment under this task this year was completion of our "Bibliography of aquatic ecosystem effects, analytical methods and treatment technologies for organic compounds in advanced fossil-fuel processing effluents" and submission to the EPA for publication. The bibliography contains 1314 citations which are indexed by chemicals, keywords, taxa and authors.
- Toward completing the second volume of the bibliography, we transcribed from cards to computer storage in excess of 2000 citations from our laboratory literature files. These citations originated from manual searches and from updates of our original computerized literature searches used in producing the first bibliography volume.
- Computer programs used in production of the first bibliography volume were modified to accommodate format changes required for citations entered in computer storage for the second volume. These modified programs were tested with short bibliographic files.
- Citations entered into computer storage are being sorted for those to be included in the bibliography. We estimate that the second bibliography volume will contain approximately 1500 citations and will be completed in February.

Task 2: Process-Water Screening

Chemical Characterization

- We compiled from several laboratories inorganic characterizations of 19 process waters: LETC SOA-75-268 R-13 dump 8, LETC ES-77-153 drum 7, LETC ES-77-153 drum 28, LETC ES-78-030 S-54 and LETC ES-78-036 S-55 simulated in situ oil-shale retort waters; Geokinetics-9, Paraho Tank-491 (1975-1976 composite), Paraho Tank-500 (1977-1978 composite), Occidental-6 and Lawrence Livermore oil-shale retort waters; Occidental-6 oil-shale condenser water; Paraho Year-1, Year-2 and Year-3 simulated spent oil-shale leachate waters; Vernal TS-2C tar-sands process wastewater; and Hanna-3, Hanna-4B OlW and Lawrence Livermore Hoe Creek underground coal gasification condenser waters. These process waters were then compared to a published summary of the analyses from 18 simulated in situ oil-shale retort waters.

-We also conducted reverse-phase high-pressure liquid chromatographic gradients of all of the above process waters plus the Hanna-4B 02W and Hanna-4B 03W post-gasification backflood condensate waters, and the Grand Forks Lurgi process tar-saturated water. These chromatograms are presented and discussed.

Toxicity Studies

-We completed this year 96-hr flow-through toxicity bioassays with fathead minnows and rainbow trout and 48-hr flow-through bioassays with Daphnia pulicaria exposed to 5 oil-shale process waters, 1 tar-sand process water, 2 underground coal gasification (UCG) condenser waters, 1 post-gasification backflood condenser water, as well as 2 bioassays with fossil-fuel process water constituents. The LC₅₀ toxicity values for these respective species when exposed to the following oil-shale retort waters were 0.071%, 0.068% and 0.147% for Paraho Tank-500 1977-1978 composite, 0.64%, 0.52% and 3.0% for Paraho Tank-491 1975-1976 composite, 0.173%, 0.088% and 0.153% for Lawrence Livermore, and 0.65%, 0.35% and 0.89% for Occidental-6; when exposed to Occidental-6 oil-shale condenser water were 0.193%, 0.176% and 0.193%; when exposed to Vernal TS-2C composite for tar-sands process water were >3.0%, 1.5% and >3.0%; when exposed to the following UCG condenser waters were 0.21% and 0.082% (no value determined for Daphnia) for Lawrence Livermore Hoe Creek and 0.33%, 0.083% and 0.37% for Hanna-4B 01W; when exposed to Hanna-4B 02W UCG post-gasification backflood water condensate were 1.4%, 0.68% and >2.0%; when exposed to ammonia (NH₃) were 1.59 mg/l, 0.77 mg/l and 1.16 mg/l; and when exposed to ammonia plus phenol dosed at the concentration of Hanna-3 UCG condenser water (63 mg/l NH₄⁺ and 6.9 mg/l phenol) were 0.21%, and 0.11% and 0.26%.

-LC₅₀ values for fathead minnow larvae and D. pulicaria in static 24-hr acute bioassays were determined for (LC₅₀ values for respective species) Vernal TS-2C composite tar-sands process water (0.6%, 0.87%); Grand Forks Lurgi process coal gasification tar saturated water (1.55%, 2.85%); Paraho spent oil-shale leachates from simulated Year 1, Year 2 and Year 3 annual precipitations (no observed toxicity); Paraho Tank-491 1975-1976 composite oil-shale retort water (0.9, 2.85%), Paraho Tank-500 1977-1978 composite oil-shale retort water (<0.05%, 0.079%); 5 LETC aboveground simulated in situ oil-shale retort waters: SOA-75-268 R-13 (0.071%, 0.134%), ES-77-153 Drum 7 (0.165%, 0.285%), ES-77-153 Drum 28 (0.10%, 0.245%), ES-78-30 S-54 (0.20%, 0.285%) and ES-78-036 S-55 (0.48%, 0.85%); and Lawrence Livermore Hoe Creek post-gasification groundwater Well A in gasification zone (6.7%, >15%) and Well 6 outside gasification zone in direction of aquifer flow (3.0%, >10%).

-Larval fathead minnows and Daphnia pulicaria were also exposed in static 24-hr bioassays to fractions of Omega-9 and Geokinetics-9 oil-shale retort water. The hydrophilic fractions of both retort waters were the most toxic; essentially all of the ammonia found in the Omega-9 fractions were in the hydrophilic fractions.

- Bioassays determining the avoidance/preference response of rainbow trout and fathead minnows to phenol and of rainbow trout to Paraho Tank-500 1977-1978 composite oil-shale retort water were completed this year. Neither results from the bioassays exposing fathead minnows to phenol nor results from exposures of rainbow trout to Paraho retort water have been statistically analyzed yet. However, the results from 3 separate avoidance/preference tests with rainbow trout in phenol solutions suggest that the threshold avoidance level lies somewhere between 3.2 and 6.5 mg/l phenol. (Last year we determined the 96-hr LC₅₀ value for rainbow trout exposed to phenol to be 8.9 mg/l and the maximum acceptable toxicant concentration to be 0.1 mg/l.)
- In embryo-larval bioassays, fathead minnow growth was significantly reduced by 0.0075% Occidental-6 oil-shale retort water and by 0.0016% Paraho Tank-500 1977-1978 composite oil shale retort water. Hatchability and survival were affected by higher concentrations of both process waters. Rainbow trout growth was significantly reduced by 0.20 mg/l phenol; because only 2 tanks of fry were reared in each phenol concentration, we were not able to statistically determine significant differences in survival.

Degradation Studies

- The degradation studies of Omega-9 and Geokinetics-9 oil-shale retort waters inoculated with oil-refinery settling pond water were terminated after 180- and 140-day HPLC analyses, respectively. Despite differences in numbers and concentrations of organic compounds in these process waters, the degradation trends for both process waters were similar with a general pattern of peak reductions throughout the chromatograms and peak migrations from the less polar to more polar regions of the chromatograms. Five persisting chromatographic peaks from the 130-day Omega-9 samples were collected and sent to R. K. Skogerboe's laboratory at Colorado State University for identification; the results of these analyses are pending.
- Our second degradation study, in which Geokinetics-9 retort water was inoculated with water from 2 sites on the Laramie River and the oil-refinery settling pond, was terminated after 65 days when the environmental chamber malfunctioned. While the data from this study is of a preliminary nature, it indicates that microbial populations from natural surface waters do have the abilities to degrade several organic constituents found in waters effluent from such processes.
- In our final set of degradation experiments completed this year, we exposed Occidental-6 oil-shale condenser and retort waters and Paraho Tank-500 1977-1978 composite oil-shale retort water to microbial population in waters from 2 sites on the Laramie River and the oil-refinery settling pond. While analyses of the results from these studies are still in progress, comparisons of interpretive tracings from the companion chromatograms of the 3 process waters inoculated with oil-refinery settling pond microbial communities indicate the

existence of 3 classes of chromatographic peaks: (1) those persistent in all 3 of the process waters, (2) those persistent in some and degraded in others, and (3) those degraded in all 3 of the process waters. The significance of these classes is discussed.

Bioaccumulation Studies

- The Veith and Kiwus exhaustive steam distillation method was adapted for extraction of aromatic and polynuclear aromatic compounds. Five different reservoir solvent systems were tested for extraction efficiencies with at least 1 of 3 different ^{14}C -labeled aromatic compounds. The compounds were extracted from spiked water samples or fish exposed to the compounds, with 16 hr of distillation, 75% n-butyl ether/20% cyclohexane/5% n-octanol provided satisfactory extracting efficiencies for most test extractions: 70% for phenol in spiked solution, 99% for naphthalene in spiked solution, 77% for benzo(a)pyrene in spiked solution and 99% for naphthalene from exposed fish. However, extraction efficiencies from benzo(a)pyrene exposed fish were <1%; perhaps due to lipid binding of B(a)P. Total recovery of the ^{14}C -compounds for all experiments was >90%.
- Using exhaustive steam distillation procedures with surviving fathead minnows exposed in 5 naphthalene concentrations of an acute toxicity bioassay provided 96-hr bioaccumulation factors ranging from 241 to 377. In comparison, the 48-hr bioaccumulation factors calculated for ^{14}C -labeled compound exposures from the bioaccumulation studies were 7 for phenol, 26 for p-cresol, 182 for naphthalene and 177 for benzo(a)pyrene.

Task 3: Methods Development

Bacterial Bioluminescence Assay

- This year we established and improved a bacterial bioluminescence assay for measuring toxicity of process waters and process-water constituents from advanced fossil-fuel conversion technologies.
- Using the assay, we determined EC_{50} values (the concentration of toxicant inhibiting bacterial bioluminescence by 50%) for 11 oil-shale process waters, 2 in situ coal gasification groundwater samples, 1 tar-sands process water, fractions from 2 oil-shale retort waters, and a series of inorganic and organic constituents from advanced fossil-fuel technology process waters.
- EC_{50} values were compared to LC_{50} values from static and flow-through bioassays with fish species. For most comparisons EC_{50} values were within a factor of 2 of the corresponding fish LC_{50} , indicating that the bacterial system could be a valuable tool for routinely monitoring the toxic properities of effluent streams.

Task 4: Recommendations

-As of the end of the Year-3 funding period we present estimated safe concentration (ESC) values for 6 oil-shale retort waters, 1 oil-shale condenser water, 3 UCG condenser waters, 1 UCG post-gasification backflood water condensate, 1 tar-sand extraction process water, and 13 process-water constituents.

2.0 INTRODUCTION

This is the third annual progress report, covering the Year 3 funding period, under Task Agreement #001, DOE-UWRMIEE Contract #DE-AS20-79-LC-01761, a project to evaluate the effects of aqueous effluents from in situ fossil fuel processing technologies on aquatic biota. The project is funded jointly by the U.S. Environmental Protection Agency through the Environmental Research Lab-Duluth and the U.S. Department of Energy through the Laramie Energy Technology Center.

Briefly, the goals of the project are:

1. Evaluate the toxicity of process water effluents on aquatic biota;
2. Recommend maximum exposure concentrations for process water constituents; and
3. Assist DOE in using project data and recommendations to design control technologies and to assess environmental impacts.

The project objectives for Year 3 were pursued through 4 tasks:

1. Literature reviews on process water constituents, possible environmental impacts and potential control technologies.
2. Process water screening through chemical characterization, toxicity, degradation and bioaccumulation studies.
3. Methods development and evaluation of microbial bioluminescence toxicity assays, double solvent extraction for exhaustive steam distillation of fish tissue containing xenobiotic compounds, and degradation assays for organics in fossil-fuel process waters.
4. Recommendations on maximum exposure concentrations for process water constituents based on data from the project and from the literature.

In the remainder of this report, progress toward completion of the project goals is presented in sections on Administration, Task Reports, and Additional Activities.

3.0 ADMINISTRATION

3.1 Staff

The following individuals worked part or fulltime on the various project tasks during this year (see Section 4.0 TASK REPORTS for those currently working and their task assignments).

- Harold L. Bergman (Principal Investigator)
- A. Duane Anderson (CoInvestigator)
- Michael Parker (CoInvestigator)
- G. Michael DeGraeve (Research Associate)
- M. E. Lebsack (Research Associate)
- Dianne Geiger (Research Assistant)
- Rodney Johnson (Research Assistant)
- Joan Anderson (Scientist I)
- Joe Inkel (Scientist I)
- Michael Marcus (Scientist I)
- Deborah Sanchez (Scientist I)
- Andrea Denny (Technician)
- Robert Elder (Technician)
- Erica Heath (Technician)
- Cindy Jones (Technician)
- Joseph Meyer (Technician)
- Ron Overcast (Technician)
- Dennis Woods (Technician)
- Linda Zeveloff (Technician)

3.2 Staff Meetings and Seminars

During this year we held monthly staff meetings to review progress, present results and conclusions for staff criticism, and plan upcoming work.

3.3 Administrative Problems

Discussions are in progress with the Project Management Team, representing the U.S. Department of Energy and the Environmental Protection Agency, for continuation of this project beginning on 1 January 1980.

4.0 TASK REPORTS

4.1 Task 1: Literature Review (Marcus, Zeveloff, Parker, Bergman)

4.1.1 Work Completed This Year

Our major accomplishment under this project task this year was completion and submission for publication of the "Bibliography of aquatic ecosystem effects, analytical methods and treatment technologies for organic compounds in advanced fossil-fuel processing effluents." In the bibliography there are 354 pages and 1314 citations which are indexed by chemicals, keywords, taxa and authors. To index the chemicals 2803 terms were used, 1218 terms for the keyword index, 1326 groupings for the taxa index, and 2440 author names. These terms were keyed in the respective computer files used in indexing. By such keying, synonymous terms and those terms to be printed in the bibliography were identified. For example, all citations pertaining to degradation, degradability, decomposition, etc., were indexed under degradation; the term degradability did not appear in the index, whereas decomposition was cross referenced: "decomposition (see degradation)."

Submission of the bibliography for publication was delayed until mid year primarily because of problems indexing citations. The Xerox TEXT processing system, through which we intended to index the bibliography, was found to be so inefficient for our purposes as to make indexing impossible. To investigate the possibility of contracting the indexing to another computer facility, we contacted various organizations which produce bibliographies, including the Technical Information Center at Oak Ridge. No facility was found which could index the bibliography to our rigorous specifications. Therefore, Duane A. Stoller and other personnel of the University of Wyoming's Division of Computer Services wrote a series of programs to perform the appropriate indexing, sorting and editing operations required for the index.

Copies of the final computer printed versions for each of the first pages from the citations and the 4 bibliographic indexes are presented in Exhibits 1 through 5. The bibliography is based on a literature search of nearly 500 chemicals found in products and process waters from advanced fossil-fuel technologies. Of these 500 chemicals only about 40% had relevant published information included in the bibliography. This small percentage indicates the lack of knowledge on potential fates and effects for many chemical compounds accompanying process wastes from oil-shale, coal and tar-sands recovery operations. As such the bibliography is of use not only in leading investigators to finding what is known about various chemical compounds, but also in identifying those compounds on which little is known of their environmental fates and effects. Toward this dual purpose, we are compiling a second volume of the bibliography to be completed early in the fourth year of this project.

Whereas the first bibliographic volume was compiled solely from computerized literature searches, the second volume is compiled from both computerized searches and manual searches. We have transcribed

<1>

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BAKU. USSR. LG EN.
YR 72. DE SPOROPOLLENIN PYROLYSIS IONENE
TI DETERMINATION OF STYRENE IN WASTE NAPHTHALENE. POLLEN SPOROPOLLENIN
WATERS FROM THE COPREPARATION OF PYROLYSIS. SHALE KEROGEN
PROPYLENE OXIDE AND STYRENE BY A SPOROPOLLENIN PYROLYSIS. COAL
DERIVED POLAROGRAPHIC METHOD. SPOROPOLLENIN PYROLYSIS.
SO AZERB. KHIM. ZH. P169-72. 1972. AN C 08025142139.
LG RS.
DE STYRENE DETN WASTE WATER. WASTE
WATER ANALYSIS STYRENE.
AN C 07902009477.

<2>

- AU ABBOTT B J. GLEDHILL W E.
YR 71.
TI THE EXTRACELLULAR ACCUMULATION OF
METABOLIC PRODUCTS BY
HYDROCARBON-DEGRADING
MICROORGANISMS.
SO ADV APPL MICROBIOL. 14. P249- 388.
1971.
LG EN.
IJ BACTERIA: ME.
MN ACIDS: BI. BENZENE: ME. ETHERS-
CYCLIC: BI. KETONES: BI. OXYGEN-
CONSUMPTION. TERPENES: ME.
AN M 72117440.

<3>

- AU ABIGNENTE, ENRICO.
IN IST. CHIM. FARM. TOSSICOL. UNIV.
NAPOLI. NAPLES. ITALY.
YR 68.
TI CURRENT ASPECTS IN THE TOXICOLOGICAL
CHEMISTRY OF PHENOLS.
SO BILL. CHIM. FARM. 107. P269-86; (6),
333-52. 1968.
LG IT.
DE REVIEW PHENOLS TOXICOL. PHENOLS
TOXICOL REVIEW. TOXICOL PHENOLS
REVIEW. WATER PHENOLS REVIEW. ATM
PHENOLS REVIEW. FOODS PHENOLS
REVIEW.
AN C 07309043328.

<4>

- AU ACHARI, R. G. SHAW, G. HOLLEYHEAD,
R.
IN SCH. CHEM. UNIV. BRADFORD. BRADFORD.
ENGL.
YR 73.
TI IDENTIFICATION OF IONENE AND OTHER
CAROTENOID DEGRADATION PRODUCTS FROM
THE PYROLYSIS OF SPOROPOLLENINS

<5>

- AU ACKERMAN, D. G. CLAUSEN, J. F.
JOHNSON, R. J. ZEE, C. A.
IN TRW DEFENSE AND SPACE SYSTEMS GROUP,
REDONDO BEACH, CALIF. ENVIRONMENTAL
PROTECTION AGENCY, WASHINGTON, D.C.
OFFICE OF SOLID WASTE MANAGEMENT
PROGRAMS. 409 637.
YR 77. 98P.
TI DESTROYING CHEMICAL WASTES IN
COMMERCIAL SCALE INCINERATORS.
FACILITY REPORT NUMBER 3 - SYSTEMS
TECHNOLOGY.
NT FINAL REPT.
PR PC A05/MF A01.
AB INCINERATION TESTS OF SELECTED
CHEMICAL WASTES WERE CONDUCTED AT
THE FLUIDIZED BED INCINERATOR
FACILITY OPERATED BY BLACK CLAWSON
FIBRECLAIM, INC. IN FRANKLIN, OHIO.
THESE TESTS WERE PERFORMED UNDER
CONTRACT WITH SYSTEMS TECHNOLOGY
CORPORATION TO DETERMINE THE
EFFECTIVENESS OF THERMAL DESTRUCTION
OF TWO DIFFERENT INDUSTRIAL LIQUID
WASTES: (1) AN AQUEOUS PHENOL
SLUDGE, AND (2) AN AQUEOUS SOLUTION
OF METHYL METHACRYLATE MONOMER.
EACH WASTE WAS BURNED AT TWO
DIFFERENT CONDITIONS TO DETERMINE
THE EFFECTS OF NORMAL OPERATING AND
EQUIPMENT VARIABLES. ANALYSIS OF
COMBUSTION GAS SAMPLES INDICATED
DESTRUCTION EFFICIENCIES OF OVER
99.999 PERCENT FOR EACH WASTE
CONSTITUENT. STANDARD EPA METHOD 5
TESTS WERE PERFORMED ON STACK
EMISSIONS TO DETERMINE PARTICULATE
LOADING AND COMPOSITION. NO
ORGANICS WERE DETECTED IN THE
SCRUBBER WATER OR ASH. TEST RESULTS
INDICATED THAT EITHER WASTE CAN BE
EFFECTIVELY DESTRUCTED IN A
FLUIDIZED BED REACTOR. ESTIMATED
COST OF DESTROYING 13.2 MILLION
LITERS/YEAR OF AQUEOUS METHYL
METHACRYLATE WAS \$6 MILLION CAPITAL
INVESTMENT AND AN OPERATING COST OF

Exhibit 2. Copy of the first page of "Chemical Index."

ACENAPHTHALENE (SEE ACENAPHTHENE)	(NO CITATIONS)
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ACETAMIDE 470	ALLYL CHLORIDE (SEE 1-CHLORO-2-PROPENE)
ACETENE (SEE ETHYLENE)	ALNUSANE (NO CITATIONS)
ACETIC ACID 184, 197, 281, 342, 380, 466, 470, 484 954, 1093, 1113, 1208, 1209, 1281, 1306	ALPHA-BUTYLENE (SEE BUTENE)
ACETIC ALDEHYDE (SEE ACETALDEHYDE)	ALPHA-COLLIDINE (NO CITATIONS)
ACETONITRILE 105, 428, 470, 1151	ALPHA-GAMMA-BUTADIENE (SEE 1,3-BUTADIENE)
ACETOPHENONE 67, 401, 612, 794, 852	ALPHA-METHYLBENZYL ALCOHOL (NO CITATIONS)
ACETOXYBUTENE (NO CITATIONS)	ALPHA-METHYLBUTYRONITRILE (NO CITATIONS)
ACETYLENE (SEE ETHYNE)	ALPHA-METHYLNAPHTHALENE (SEE 1-METHYLNAPHTHALENE)
ACETYLENE TETRACHLORIDE (SEE 1,1,2,2-TETRACHLOROETHANE)	ALPHA-METHYLPYRIDINE (SEE 2-METHYLPYRIDINE)
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ACTIVATED CARBON (SEE WASTE TREATMENT, CARBON FILTRATION)	ALCOHOL OXIDOREDUCTASES 70, 119, 409, 490, 498, 1007, 1008
ACTIVATED SLUDGE (SEE WASTE TREATMENT, SLUDGE)	ALDEHYDE OXIDOREDUCTASES 119, 289, 490
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ACUTE TOXICITY (SEE TOXICOLOGY, ACUTE)	AMINASE 694
ADAPTATION (SEE ACCLIMATION)	AMYLASE 996
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Exhibit 4. Copy of the first page of "Taxonomic Index."

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from cards to computer storage all references gathered during this project. In all, we estimate that in excess of 2000 citations have been transferred. This is in addition to the 1314 citations previously stored from the first bibliography.

To accommodate slight format changes necessary for citations entered into computer storage from our card files, we modified the computer programs used in production of the first bibliographic volume. Test runs using the revised programs with small test files of bibliography citations were completed and these programs should be debugged by the time the citation file for the second volume is ready for printing. We plan to index the second volume using essentially the same chemical keywords and taxa index terms as used in the first volume. Therefore, computer production of the second volume should consume much less time than required for the first volume.

While additional citations are still being entered into computer storage from monthly computerized search updates, we have begun to sort the earlier entries for those appropriate to the bibliography. Such a sort is necessary because the references we have accumulated during this project include not only citations pertaining to aquatic ecosystem effects, analytical methods and treatment technologies for organic compounds in advanced fossil-fuel processing effluents, but also citations pertaining to terrestrial effects, methods using compounds or for compounds not yet identified as in advanced fossil-fuel effluents, effluents from other sources, advanced fossil-fuel technologies, etc. Citations in these latter categories have provided us with insight for working within the project's goals and objectives. In making the final sort, those citations exceptionally informative or providing excellent reviews are retained for the bibliography. All citations are retained for our laboratory's computerized literature file. Currently, we estimate that the second bibliographic volume will contain approximately 1500 citations.

4.1.2 Work in Progress and Planned for Next Quarter

We are continuing to enter citations into computer storage and to sort previously entered citations for those appropriate to the bibliography. Final modifications to accommodate the new formats required for the second bibliographic volume are being made to the computer programs required producing the printed volume. We expect the second volume to be completed in February. Following printing of the second volume, we will transfer our laboratory's computerized literature files to the University's new CDS Cyber computer system from the old Xerox Sigma-7 system.

Emphasis on this task will be markedly reduced during 1980 following submission of the second volume. As in previous years, we will continue to use computerized literature searches to identify published information on aquatic ecosystem hazards (e.g., toxicity, degradation, bioaccumulation, cycling, control technology) associated with advanced fossil fuel processing effluents, process effluent fractions and important individual compounds.

4.2 Task 2: Process-Water Screening

Our process-water screening strategy consists of a series of aquatic hazard tests on whole process waters and process-water fractions; the tests are arranged in a three-level screening system which has as its principal components chemical characterization, toxicity, degradation and bioaccumulation studies (Fig. 4.2-1). Results obtained from the Level 1 studies determine whether to conduct Level 2 studies; similarly Level 2 studies are the criteria determining advancement to Level 3 studies.

Process waters that we have thus far obtained and our progress in screening these waters are summarized in Table 4.2-1. Detailed discussions of the work completed this year and that planned for next year are presented in the following sections.

4.2.1 Chemical Characterization (Meyer, Sanchez, Marcus, Bergman)

4.2.1.1 Work Completed This Year

We compiled from several laboratories inorganic characterizations of the following 19 process waters (Tables 4.2.1-1 to 4.2.1-4):

Simulated in situ oil-shale retort waters--LETC SOA-75-268 R-13 dump 8, LETC ES-77-153 drum 7, LETC ES-77-153 drum 28, LETC ES-78-030 S-54 and LETC ES-78-036 S-55;

Oil-shale retort waters--Geokinetics-9, Paraho Tank-491 (1975-1976 composite), Paraho Tank-500 (1977-1978 composite), Occidental-6 and Lawrence Livermore;

Oil-shale condenser water--Occidental-6;

Simulated spent oil-shale leachate waters--Paraho Year 1, Paraho Year 2 and Paraho Year 3;

Tar-sands wastewater--Vernal TS-2C; and

Coal gasification condenser waters--Hanna-3 UCG, Hanna-4B OlW UCG and Lawrence Livermore Hoe Creek UCG.

For the 5 simulated in situ oil-shale retort waters and the tar sands water, analyses were performed for the Laramie Energy Technology Center (LETC) by the Wyoming Department of Agriculture's Division of Laboratories, Laramie (WDA) and by CDM/Accu-Labs of Wheatridge, Colorado (CDM). Mr. Tom Spedding of LETC supplied these values to us. WDA also analyzed the 5 oil-shale retort waters, the oil-shale condenser water and the 3 coal gasification waters. Dr. Rodney Skogerboe's group at Colorado State University (CSU) quantified major elements present in the 3 oil-shale leachates, while we determined several additional water chemistry parameters. Also, we have included in Table 4.2.1-3 the Omega-9 oil-shale retort water analyses published by Fox et al. (1978).

Figure 4.2-1. Strategy for process-water screening.

Table 4.2-1. Summary of progress with process-water screening as of 15 December 1979 (NA = water for test is not available; TU = preceding screening level indicates test is unnecessary).

Process Water	Chemical characterization ^a			Toxicity bioassay				Degradation	Bioaccumulation
	Water Chemistry	HPLC gradient analysis	Other	24-hr static acute	96-hr flo-thru acute	Embryo-larval chronic	Behavior		
<u>Coal gasification water</u>									
Hanna-3 condenser water	UW	UW		completed	completed	completed	NA	NA	NA
Grand Forks Lurgi process tar-saturated water	TU	UW		completed	NA	NA	NA	NA	NA
Hoe Creek	WDA	UW		completed	completed	NA			
Hanna-4B 01W	WDA	UW		completed	completed				
<u>Post-gasification backflood condensate</u>									
Hanna-4E 02W	TU	UW		completed	completed	TU	TU	TU	TU
Hanna-4B 03W	TU	UW		completed	TU	TU	TU	TU	TU
<u>Post-gasification ground water</u>									
Hoe Creek Well A			LLL	completed	TU	TU	TU	TU	TU
Hoe Creek Well 6			LLL	completed	TU	TU	TU	TU	TU
<u>Pre-retort oil shale ground water</u>									
RS-12		TU		completed	TU	TU	TU	TU	TU
<u>Oil shale retort water</u>									
Omega-9 - whole water	UW	UW	Fox et al. (1978) Pellizzari (1978)	completed	completed	completed	TU	completed	
Omega-9 - fractions		TU		completed	NA	NA	NA	NA	NA
Geokinetics-9 - whole water	UW,WDA	UW		completed	completed	TU	TU	completed	
Geokinetics-9 - fractions		TU		completed	NA	NA	NA	NA	NA
Paraho Tank-491 (1975-76 composite)	WDA	UW		completed	completed	TU	TU	TU	TU
Paraho Tank-500 (1977-78 composite)	WDA	UW		completed	completed	completed	completed	in progress	
Lawrence Livermore	WDA	UW		completed	completed	TU			
Occidental-6	WDA	UW		completed	completed	completed		in progress	
<u>Oil shale condenser water</u>									
Occidental-6	WDA	UW		completed	completed	TU	TU	in progress	
<u>Simulated in situ oil shale retort water</u>									
LETC SOA-75-268 R-13	WDA,CDM	UW		completed	TU	TU	TU	TU	TU
LETC ES-77-153 Drum 7	WDA	UW		completed	TU	TU	TU	TU	TU
LETC ES-77-153 Drum 28	WDA	UW		completed	TU	TU	TU	TU	TU
LETC ES-78-30 S-54	WDA,CDM	UW		completed	TU	TU	TU	TU	TU
LETC ES-78-036 S-55	WDA,CDM	UW		completed	TU	TU	TU	TU	TU

Table 4.2-1. (Continued)

Process Water	Chemical characterization ^a			24-hr static acute	96-hr flow-thru acute	Embryo- larval chronic	Behavior	Degradation	Bioaccumulation
	Water chemistry	HPLC gradient analysis	Other						
<u>Spent oil shale leachate</u>									
Paraho-simulated annual precipitation:									
Year 1	UW,CSU	UW		completed	TU	TU	TU	TU	TU
Year 2	UW,CSU	UW		completed	TU	TU	TU	TU	TU
Year 3	UW,CSU	UW		completed	TU	TU	TU	TU	TU
<u>Tar sands water</u>									
Vernal TS-2C composite (ES-77-206)	WDA,CDM	UW		completed	completed	TU	TU	TU	TU

^a UW = analyses performed in our laboratory; WDA = analyses performed by the Wyoming Department of Agriculture, Division of Laboratories, Laramie; CSU = analyses performed by Dr. R. Skogerboe's group at Colorado State University, Fort Collins; CDM = analyses performed by CDM/Accu-Labs of Wheatridge, Colorado.

Table 4.2.1-1. Water quality characteristics of simulated in situ oil-shale retort waters.

Water quality parameter ^a	LETC SOA-75-268 R-13 dump 8	LETC ES-77-153 drum 7	LETC ES-77-153 drum 28	LETC ES-78-030 S-54	LETC ES-78-036 S-55
Alkalinity, total (CaCO ₃)	-- ^b	--	--	--	--
Biological oxygen demand (5 day)	--	--	--	--	--
Carbon					
Bicarbonate (HCO ₃ ⁻)	36,000	32,000	33,000	0	8,700
Carbonate (CO ₃ ⁼)	9,500	24,000	21,000	0	0
Dissolved organic carbon	--	--	--	--	--
Total organic carbon	3,798	--	--	--	--
Chemical oxygen demand	--	9,900	10,000	17,000 ^b	270
Chloride	5,800	2,800	2,900	*	2,400
Conductance (μmhos/cm)	22,400	20,200	17,300	20,000	26,400
Cyanide	--	--	--	--	--
Fluoride	270	29	31	*	130
Hardness (CaCO ₃)	30	55	51	1,000	88
Metals					
Arsenic	3.7	3.5	7.5	0.59	1.0
Aluminum	0.12	--	--	17	0.04
Barium	0.042	--	--	0.26	0.2
Beryllium	<0.001	--	--	0.006	<0.001
Boron	0.7	*	*	1.1	0.4
Cadmium	0.001	<0.1	<0.1	0.069	<0.006
Calcium	3.7	2.4	2.1	300	12
Chromium	0.012	--	--	0.23	0.02
Copper	0.008	--	--	0.28	0.06
Iron	21	--	--	1,100	5
Lead	0.021	--	--	0.31	0.2
Magnesium	5.0	12	11	69	14
Manganese	0.17	--	--	33	0.4
Mercury	<0.003	0.038	0.041	<0.001	0.44
Molybdenum	0.21	--	--	0.017	0.5
Nickel	0.14	--	--	0.094	0.8
Potassium	47	3.6	2.7	16	27
Selenium	0.038	1.0	0.90	<0.005	0.2
Silver	<0.001	--	--	<0.001	0.002
Sodium	180	330	340	18	560
Strontium	0.032	--	--	1.0	0.1
Zinc	0.36	--	--	5.5	0.1

Table 4.2.1-1. (Continued)

Water quality parameter ^a	LETC SOA-75-268 R-13 dump 8	LETC ES-77-153 drum 7	LETC ES-77-153 drum 28	LETC ES-78-030 S-54	LETC ES-78-036 S-55
Nitrogen					
Ammonia (total ammonia as NH ₃)	7,900	10,000	8,000	5,500	1,500
Ammonium (total ammonia as NH ₄ ⁺)	8,400	11,000	8,500	5,900	1,600
Nitrate (NO ₃ ⁼)	--	*	*	*	*
Nitrite (NO ₂ ⁻)	--	--	--	--	--
Total Kjeldahl (N)	6,600	5,700	4,800	7,500	4,400
pH (units)	8.9	9.4	9.5	3.5	8.5
Phenols	--	--	--	--	--
Phosphorous					
Total orthophosphate (PO ₄ ⁼)	--	--	--	--	--
Silica	87	--	--	--	--
Solids					
Suspended	--	--	--	--	--
Total	--	--	--	--	--
Total dissolved	18,400	3.1	3.8	372	20,700
Volatile	10,700	--	--	--	--
Sulfur					
Sulfate	1,700	790	890	17,000	4,800
Sulfide	--	--	--	--	--
Institutions performing analyses ^c	WDA, CDM	WDA	WDA	WDA, CDM	WDA, CDM

^aValues are mg/l unless otherwise noted.

^b-- = analyses not completed; * = analyses not completed because of interfering compounds.

^cCDM = CDM/Accu-Labs of Wheatridge, Colorado; WDA = Wyoming Department of Agriculture, Division of Laboratories, Laramie.

Table 4.2.1-2. Water quality characteristics of oil-shale retort waters.

Water quality parameter ^a	Geokinetics-9 oil-shale retort water	Paraho Tank-491 oil-shale retort water	Paraho Tank-500 oil-shale retort water	Lawrence Livermore oil-shale retort water	Occidental-6 oil-shale retort water
Alkalinity, total (CaCO ₃)	9,500	1,500	23,000	22,000	6,700
Biological oxygen demand (5 day)	-- ^b	--	--	--	--
Carbon					
Bicarbonate (HCO ₃ ⁻)	8,000	1,800	28,000	2,800	6,100
Carbonate (CO ₃ ⁼)	1,800	0	0	12,000	1,000
Dissolved organic carbon	--	--	--	--	--
Total organic carbon	--	--	--	--	--
Chemical oxygen demand	6,200	17,000	140,000	8,100	7,900
Chloride	* ^b	*	*	1,600	350
Conductance (µmhos/cm)	13,800	6,470	63,500	29,700	12,800
Cyanide	2.6	1.1	0.19	0.95	<0.001
Fluoride	1.2	13	70	1.1	45
Hardness (CaCO ₃)	280	1,200	280	140	11
Metals					
Arsenic	0.47	43	20	1.1	0.7
Aluminum	<0.1	<0.1	0.2	<0.5	<0.5
Barium	0.5	0.3	<0.5	<0.1	<0.2
Beryllium	--	--	--	--	--
Boron	78	17	11	0.2	100
Cadmium	0.05	<0.05	0.14	<0.01	<0.01
Calcium	4.9	200	16	6.9	1.9
Chromium	<0.1	0.02	0.10	<0.1	<0.1
Copper	<0.1	<0.1	0.13	0.03	<0.01
Iron	1.3	1.6	1.6	0.5	0.2
Lead	0.6	<0.1	<0.1	<0.1	<0.1
Magnesium	64	170	58	30	1.6
Manganese	0.08	0.44	0.09	0.06	0.05
Mercury	<0.001	0.17	0.035	<0.1	<0.1
Molybdenum	--	--	--	--	--
Nickel	0.2	<0.1	1.2	0.2	<0.1
Potassium	9.0	8.2	38	13	60.0
Selenium	3.1	0.85	<0.005	0.01	0.02
Silver	<0.05	<0.5	<0.05	<0.5	<0.5
Sodium	5,300	430	310	130	330
Strontium	--	--	--	--	--
Zinc	0.51	<0.05	400	0.1	0.08

Table 4.2.1-2. (Continued)

Water quality parameter ^a	Geokinetics-9 oil-shale retort water	Paraho Tank-491 oil-shale retort water	Paraho Tank-500 oil-shale retort water	Lawrence Livermore oil-shale retort water	Occidental-6 oil-shale retort water
Nitrogen					
Ammonia (total ammonia as NH ₃)	1,900	190	29,000	11,000	1,000
Ammonium (total ammonia as NH ₄ ⁺)	2,000	200	31,000	12,000	1,000
Nitrate (NO ₃ ⁻)	*	*	*	*	*
Nitrite (NO ₂ ⁻)	0.0	0.0	0.0	*	*
Total Kjeldahl (N)	1,800	1,500	28,000	10,000	1,000
pH (units)	8.8	8.2	8.3	9.1	8.9
Phenols	6,800	0.05	*	*	*
Phosphorous					
Total orthophosphate (PO ₄ ⁼)	*	*	*	*	*
Silica	310	0.2	60	9.2	*
Solids					
Suspended	20.0	36	86	32	100
Total	15,900	8,650	111,000	1,330	10,390
Total dissolved	15,900	8,610	111,000	1,270	10,200
Volatile	2.0	24	82	28	92
Sulfur					
Sulfate	1,600	1,400	3,200	2,300	1,400
Sulfide	<0.5	0.9	0.1	88	<0.025
Institutions performing analyses ^c	WDA	WDA	WDA	WDA	WDA

^a Values are mg/l unless otherwise noted.

^b -- = analyses not completed; * = analyses not completed because of interfering compounds.

^c WDA = Wyoming Department of Agriculture, Division of Laboratories, Laramie.

Table 4.2.1-3. Water quality characteristics of oil-shale retort, oil-shale condenser and oil-shale leachate waters.

Water quality parameter ^a	Omega-9 oil-shale retort water	Occidental-6 oil-shale condenser water	Paraho simulated oil-shale leachate; Year 1	Paraho simulated oil-shale leachate; Year 2	Paraho simulated oil-shale leachate; Year 3
Alkalinity, total (CaCO ₃)	16,200	22,000	61	61	55
Biological oxygen demand (5 day)	740	--	--	--	--
Carbon					
Bicarbonate (HCO ₃ ⁻)	15,940	19,000	0	0	0
Carbonate (CO ₃ ⁼)	500	3,300	28	28	24
Dissolved organic carbon	-- ^b	--	--	--	--
Total organic carbon	1,003	--	--	--	--
Chemical oxygen demand	8,100	2,800	--	--	--
Chloride	824	210	--	--	--
Conductance (µmhos/cm)	20,400	27,200	1,180	825	415
Cyanide	0.42	0.06	--	--	--
Fluoride	60	0.0	--	--	--
Hardness (CaCO ₃ ⁻)	110	18	272	270	241
Metals					
Arsenic	1.0	0.05	--	--	--
Aluminum	<0.03	<0.5	0.16	0.25	0.19
Barium	0.71	<0.1	0.23	0.28	0.25
Beryllium	<0.006	--	<0.025	<0.025	<0.025
Boron	27	0.0	0.22	0.31	0.19
Cadmium	0.0016	<0.01	<0.025	<0.025	<0.025
Calcium	12	6.9	100	130	60
Chromium	0.02	<0.1	<0.025	<0.025	<0.025
Copper	0.10	<0.01	<0.025	<0.025	<0.025
Iron	1.2	0.0	--	--	--
Lead	0.0045	<0.1	<0.04	<0.04	<0.04
Magnesium	20	0.1	4	2	1
Manganese	0.09	<0.05	<0.05	<0.05	<0.05
Mercury	0.0003	0.02	--	--	--
Molybdenum	0.60	--	0.50	0.45	0.16
Nickel	0.06	<0.1	<0.025	<0.025	<0.025
Potassium	47	2.0	24	19	8
Selenium	0.21	0.1	--	--	--
Silver	0.003	<0.5	--	--	--
Sodium	4,333	7.0	150	95	30
Strontium	1.12	--	1.3	1.6	1.0
Zinc	0.31	<0.01	--	--	--

Table 4.2.1-3. (Continued)

Water quality parameter ^a	Omega-9 oil-shale retort water	Occidental-6 oil shale condenser water	Paraho simulated oil-shale leachate; Year 1	Paraho simulated oil-shale leachate; Year 2	Paraho simulated oil-shale leachate; Year 3
Nitrogen					
Ammonia (total ammonia as NH ₃)	3,795	9,200	--	--	--
Ammonium (total ammonia as NH ₄ ⁺)	3,470	9,300	--	--	--
Nitrate (NO ₃ ⁼)	0.17	* ^b	--	--	--
Nitrite (NO ₂ ⁻)	--	*	--	--	--
Total Kjeldahl (N)	3,420	7,000	--	--	--
pH (units)	8.65	8.7	10.4	10.4	10.4
Phenols	60	*	--	--	--
Phosphorous					
Total orthophosphate (PO ₄ ⁼)	0.08	*	--	--	--
Silica	8	*	15	18	14
Solids					
Suspended	--	16	--	--	--
Total	14,210	576	--	--	--
Total dissolved	14,210	560	--	--	--
Volatile	--	0	--	--	--
Sulfur					
Sulfate	1,990	3.3	--	--	--
Sulfide	0.0	<0.025	--	--	--
Institutions performing analyses ^c	FOX	WDA	CSU,UW	CSU,UW	CSU,UW

^aValues are mg/l unless otherwise noted.

^b-- = analyses not completed; * = analyses not completed because of interfering compounds.

^cCSU = Dr. Rod Skogerboe's group at Colorado State University, Fort Collins; FOX = Fox et al. (1978); UW = our laboratory; WDA = Wyoming Department of Agriculture, Division of Laboratories, Laramie.

Table 4.2.1-4. Water quality characteristics of tar sands wastewater and coal gasification condenser waters.

Water quality parameter ^a	Vernal TS-2C tar sands water; (ES-77-206)	Lawrence Livermore Hoe Creek UCG condenser water	Hanna-3 coal gasification condenser water	Hanna-4B 01W coal gasification condenser water
Alkalinity, total (CaCO ₃)	0 ^b	12,000	11,200	12,200
Biological oxygen demand (5 day)	--	--	--	--
Carbon				
Bicarbonate (HCO ₃ ⁻)	0	10,000	7,300	5,840
Carbonate (CO ₃ ⁼)	0	2,300	3,150	4,460
Dissolved organic carbon	--	--	--	--
Total organic carbon	--	--	--	--
Chemical oxygen demand	2,970	7,900	--	--
Chloride	57	950	--	100
Conductance (µmhos/cm)	752	16,100	--	16,100
Cyanide	--	0.13	0.05	-- ^b
Fluoride	3.6	17	--	* ^b
Hardness (CaCO ₃)	68	12	22	25
Metals				
Arsenic	0.001	0.03	--	0.082
Aluminum	0.52	<0.5	--	1.1
Barium	0.029	<0.1	--	<0.1
Beryllium	<0.001	--	--	--
Boron	0.36	0.3	--	*
Cadmium	<0.001	<0.01	--	<0.01
Calcium	14.7	3.2	2.7	3.7
Chromium	<0.001	<0.1	--	<0.1
Copper	0.064	<0.05	--	--
Iron	91	0.00	--	--
Lead	0.081	<0.2	--	<0.1
Magnesium	7.9	0.9	3.6	3.8
Manganese	1.5	<0.05	--	--
Mercury	0.0004	0.020	--	0.059
Molybdenum	0.002	--	--	--
Nickel	0.005	<0.1	--	--
Potassium	1.8	0.5	--	1.0
Selenium	0.002	0.35	--	0.26
Silver	<0.001	<0.05	--	<0.05
Sodium	2.6	39	--	7.5
Strontium	0.068	--	--	--
Zinc	0.092	<0.02	--	--

Table 4.2.1-4. (Continued)

Water quality parameter	Vernal TS-2C tar sands water; (ES-77-206)	Lawrence Livermore Hoe Creek UCG condenser water	Hanna-3 coal gasification condenser water	Hanna-4B 01W coal gasification condenser water
Nitrogen				
Ammonia (total ammonia as NH_3)	51	4,500	20,000	4,120
Ammonium (total ammonia as NH_4^+)	53	4,800	21,000	4,360
Nitrate (NO_3^-)	*	*	--	*
Nitrite (NO_2^-)	--	<0.1	--	*
Total Kjeldahl (N)	78	3,800	--	5,040
pH (units)	4.3	8.8	9.0	9.1
Phenols	--	*	--	*
Phosphorous				
Total orthophosphate (PO_4^{3-})	--	0.00	--	*
Silica	--	47	--	*
Solids				
Suspended	--	2.0	--	54
Total	--	210	--	1,270
Total dissolved	--	210	*	1,220
Volatile	--	0.0	--	50
Sulfur				
Sulfate	120	16	--	300
Sulfide	--	100	--	--
Institutions performing analyses ^c	WDA, CDM	WDA	WDA	WDA

^a Values are mg/l unless otherwise noted.

^b -- = analyses not completed; * = analyses not completed because of interfering compounds.

^c CDM = CDM/Accu-Labs of Wheatridge, Colorado; WDA = Wyoming Department of Agriculture, Division of Laboratories, Laramie.

We conducted reverse-phase high-pressure liquid chromatographic (HPLC) gradients of all these process waters. We also chromatographed 2 post-gasification backflood condensate waters (Hanna-4B 02W and Hanna-4B 03W) and the Grand Forks Lurgi process tar-saturated water (Figures 4.2.1-1 to 4.2.1-20). The gradient chromatograms of the Paraho Year-2 and Year-3 simulated oil-shale leachates are very similar to the Paraho Year-1 chromatogram and thus are not included. Because the LETC SOA-75-268 R-13, Paraho Tank-491, Paraho Tank-500 and Hanna-3 waters displayed high UV absorbances, either the injection volume or the detector sensitivity was decreased to more readily represent the large peaks (Figures 4.2.1-1, 4.2.1-8, 4.2.1-9 and 4.2.1-15). Also different for the Hanna-3 chromatogram was a 10-min gradient as opposed to a 60-min gradient used for all of the other process waters.

Several investigators previously implicated phenol and some cresols as major toxic constituents of coal processing effluents (Parkhurst, et al. 1979(a), Parkhurst, et al. 1979(b), Herbert 1962). Thus we attempted to verify the presence of phenol in many of the process waters (Table 4.2.1-5). Two chromatographic analyses were employed. First, the HPLC reverse-phase gradients were compared with gradients of phenol standards to identify possible phenol peaks. Waters containing high concentrations of suspected phenol were then chromatographed isocratically in 20% CH₃CN/H₂O to better resolve the peaks clustered around that retention time. If a peak's elution matched the isocratic phenol standard, it was tentatively identified as phenol and its concentration was quantified. Furthermore, the suspected phenol peaks in the Hanna-4B 01W and Lawrence Livermore Hoe Creek UCG condenser waters were collected and sent to the EPA Environmental Research Laboratory-Duluth for GC/MS confirmation. We have not yet received the results of these analyses and caution that the assignment of phenol concentrations in some waters for which only gradient analyses were performed is tentative.

For a comparison of process water characteristics, we have reproduced the Fox et al. (1978) summary of chemical composition for 18 simulated in situ oil-shale retort waters (Table 4.2.1-6). They reported ranges for 43 parameters and means on 20 of these parameters for which >15 reliable water analyses were available. As noted by the authors, the large variability for most parameters may have resulted from a combination of analytical problems, sample instability, and variations in processing conditions and oil-shale composition. These process waters were not identified, so we do not know if any of the simulated in situ retort waters we investigated were also included by Fox et al.

We divided the 19 process waters reported in Tables 4.2.1-1 to 4.2.1-4 into 4 categories according to their processing modes: (1) oil-shale waters, (2) spent oil-shale leachates, (3) UCG condenser waters and (4) tar sands wastewater. Ranges of water quality characteristics for the first 3 categories are included in Table 4.2.1-6. And for the oil-shale and UCG waters we also tabulated for each parameter (1) the number of waters with values determined, (2) the number of waters with values outside the range reported by Fox et al. and (3) the number of waters with values <0.1 or >10 times the mean value reported by Fox et al.

Figure 4.2.1-1. HPLC chromatogram of LETC SOA-75-268 R-13 simulated in situ oil-shale retort water at 1/5 of the normal injection volume. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-2. HPLC chromatogram of LETC ES-77-153 drum 7 simulated in situ oil-shale retort water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-3. HPLC chromatogram of LETC ES-77-153 drum 28 simulated in situ oil-shale retort water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-4. HPLC chromatogram of LETC ES-78-030 S-54 simulated in situ oil-shale retort water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-5. HPLC chromatogram of LETC ES-78-036 S-55 simulated in situ oil-shale retort water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-6. HPLC chromatogram of Omega-9 oil-shale retort water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-7. HPLC chromatogram of Geokinetics-9 oil-shale retort water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-8. HPLC chromatogram of Paraho Tank-491 (1975-1976 composite) oil-shale retort water at 1/10 of the normal detector sensitivity. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-9. HPLC chromatogram of Paraho Tank-500 (1977-1978 composite) oil-shale retort water at 1/10 of the normal detector sensitivity. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-10. HPLC chromatogram of Lawrence Livermore oil-shale retort water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-11. HPLC chromatogram of Occidental-6 oil-shale retort water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-12. HPLC chromatogram of Occidental-6 oil-shale condenser water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-13. HPLC chromatogram of Paraho-simulated spent oil-shale leachate-Year 1. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-14. HPLC chromatogram of Vernal TS-2C composite (ES-77-206) tar sands water. Retentions of 5 reference compounds are indicated above the chromatogram.

μ-C₁₈ reverse-phase
10 μl injection
10 min linear gradient
0-100% CH₃CN/H₂O
2.0 ml/min
254 nm UV; 0.1 AUFS

Figure 4.2.1-15. HPLC chromatogram of Hanna-3 UCG condenser water at 1/5 of the normal injection volume and 1/6 of the normal gradient time. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-16. HPLC chromatogram of Hanna-4B 01W UCG condenser water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-17. HPLC chromatogram of Hanna-4B 02W post-gasification backflood condensate. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-18. HPLC chromatogram of Hanna-4B 03W post-gasification backflood condensate. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-19. HPLC chromatogram of Lawrence Livermore Hoe Creek UCG condenser water. Retentions of 5 reference compounds are indicated above the chromatogram.

Figure 4.2.1-20. HPLC chromatogram of Grand Forks Lurgi coal gasification water. Retentions of 5 reference compounds are indicated above the chromatogram.

Table 4.2.1-5. Concentrations of phenol tentatively identified for various process waters.

Process water	Suspected phenol concentration (mg/l)
<u>Coal gasification water</u>	
Hanna-3 UCG condenser	2,300 ^a
Hanna-4B 01W UCG condenser	2,300 ^{b,c}
Hoe Creek UCG condenser	830 ^{b,c}
Grand Forks Lurgi	<50 ^a
<u>Post-gasification backflood condensate water</u>	
Hanna-4B 02W	200 ^b
Hanna-4B 03W	60 ^a
<u>Tar sands wastewater</u>	
Vernal TS-2C	<100 ^a
<u>Simulated in situ oil-shale retort water</u>	
LETC SOA-75-268 R-13 dump 8	<50 ^a
LETC ES-77-153 drum 7	<50 ^a
LETC ES-77-153 drum 28	<50 ^a
LETC ES-78-030 S-54	<5 ^a
LETC ES-78-036 S-55	<50 ^a
<u>Oil-shale retort water</u>	
Omega-9	<5 ^a
Geokinetics-9	<50 ^a
Paraho Tank-491	<25 ^a
Paraho Tank-500	<250 ^a
Lawrence Livermore	<25 ^a
Occidental-6	<10 ^{b,d}
<u>Oil-shale condenser water</u>	
Occidental-6	<100 ^a
<u>Simulated oil-shale leachate water</u>	
Paraho; Year 1	<1 ^a
Paraho; Year 2	<1 ^a
Paraho; Year 3	<1 ^a

^a Value determined in our laboratory from comparison of HPLC gradient chromatogram of the process water with gradient chromatogram of a phenol standard.

^b Value determined in our laboratory from comparison of HPLC isocratic chromatogram of the process water with isocratic chromatogram of a phenol standard.

^c Suspected phenol peak collected and shipped to EPA-Duluth for confirmation of identity.

^d Also determined < 10 mg/l o-cresol present.

Table 4.2.1-6. Comparison of process water chemical characteristics with those reported in the literature.

Water quality parameter ^a	Various oil-shale process waters reported by Fox, et al. (1978)		12 oil-shale process waters analysed during this project ^c				3 Paraho simulated oil-shale leachates		3 UCG condenser waters analysed during this project ^c			
	Range	Average ^b	Range	N ₁	N ₂	N ₃	Range		Range	N ₁	N ₂	N ₃
Alkalinity, total (CaCO ₃)	18,200 - 110,900		1,500 - 23,000	7	2	--	55 - 61		11,200 - 12,200	3	3	--
Biological oxygen demand (5 day)	350 - 5,500 ^d		740	1	0	--	---		---	--	--	--
Carbon												
Bicarbonate (HCO ₃ ⁻)	4,200 - 73,640		0 - 36,000	12	2	--	0		5,840 - 10,000	3	0	--
Carbonate (CO ₃ ⁼)	0 - 15,210 ^d		0 - 24,000	12	2	--	24 - 28		2,300 - 4,460	3	0	--
Total organic carbon	2,200 - 19,000	4,700	1,003 - 3,798	2	1	0	---		---	--	--	--
Chemical oxygen demand	8,500 - 43,000 ^d	18,500 ^d	270 - 140,000	11	7	1	---		7,900	1	1	0
Chloride	0.007 - 1,910 ^d		210 - 5,800	8	4	--	---		100 - 950	2	0	--
Conductance (μmhos/cm)	15,100 - 193,000	31,000	6,470 - 63,500	12	3	0	415 - 1,180		16,100	2	0	0
Cyanide	---		<0.001 - 2.6	7	0	--	---		0.05 - 0.13	2	--	--
Fluoride	0.1 - 270		0.0 - 270	11	1	0	---		17	1	0	--
Hardness (CaCO ₃)	20 - 1,500	88	11 - 1,200	12	1	2	---		12 - 25	3	1	0
Metals												
Aluminum	0.041 - 16.6		<0.03 - 17	10	1	--	0.16 - 0.25		<0.5 - 1.1	2	0	--
Arsenic	0.46 - 10		0.05 - 43	12	3	3	---		0.03 - 0.082	2	2	--
Barium	0.002 - 0.47	0.07	0.042 - 0.71	10	2	0	0.23 - 0.28		<0.1	2	0	0
Beryllium	<0.001		<0.001 - 0.006	4	1	--	<0.025		---	--	--	--
Boron	---		0.0 - 100	10	--	--	0.19 - 0.31		0.3	1	--	--
Cadmium	<0.001 - 0.005		0.001 - 0.14	12	3	--	<0.025		<0.01	2	--	--
Calcium	0.0 - 94	7.6	1.9 - 300	12	2	2	60 - 130		2.7 - 3.7	3	0	0
Chromium	0.009 - 0.08	0.015	0.012 - 0.23	10	2	1	<0.025		<0.1	2	--	--
Copper	0.003 - 160	0.019	0.008 - 0.28	10	0	1	<0.025		---	--	--	--
Iron	0.091 - 77	7.6	0.0 - 1,100	10	2	4	---		---	--	--	--
Lead	0.002 - 0.83		0.0045 - 0.6	10	0	--	<0.04		<0.1 - <0.2	2	0	--
Magnesium	3.2 - 350	22	0.1 - 170	12	2	2	1 - 4		0.9 - 3.8	3	1	1
Manganese	0.001 - 0.39	0.099	<0.05 - 33	10	3	1	<0.05		---	--	--	--
Mercury	<0.001 - 0.39 ^d		<0.001 - 0.44	12	1	--	---		0.020 - 0.059	2	0	--
Molybdenum	0.033 - 1.2		0.017 - 0.60	4	1	--	0.16 - 0.50		---	--	--	--
Nickel	0.014 - 2.6		0.06 - 0.8	10	0	--	<0.025		---	--	--	--
Potassium	8 - 120	37	2.0 - 60.0	12	3	3	8 - 24		0.5 - 1.0	2	2	2
Selenium	<0.001 - 1.7		<0.005 - 3.1	12	1	--	---		0.26 - 0.35	2	0	--
Silver	<0.001 - 0.23		<0.001 - 0.003	10	0	--	---		<0.05	2	0	--
Sodium	45 - 1,600	320	7.0 - 5,300	12	3	3	30 - 150		7.5 - 39	2	2	1
Strontium	---		0.032 - 1.12	4	--	--	1.0 - 1.6		---	--	--	--
Zinc	0.020 - 15.1	0.28	<0.01 - 400	10	2	3	---		---	--	--	--
Nitrogen												
Ammonia (total ammonia as NH ₃)	2,100 - 16,000	8,500	190 - 29,000	12	5	1	---		4,120 - 20,000	3	2	0
Ammonium (total ammonia as NH ₄ ⁺)	1,200 - 31,400	12,900	200 - 31,000	12	3	2	---		4,360 - 21,000	3	0	0
Nitrate (NO ₃ ⁼)	6.2 - 38.5		0.17	1	1	--	---		---	--	--	--
Nitrite (NO ₂ ⁻)	<3.3		0.0	3	0	--	---		<0.1	1	--	--
Total Kjeldahl (N)	6,600 - 19,500		1,000 - 28,000	12	8	--	---		3,800 - 5,040	2	2	--
pH (Units)	8.1 - 9.4	8.7	3.5 - 9.5	12	2	1	10.4		8.8 - 9.1	3	0	0
Phenols	2.2 - 169 ^d		0.05 - 6,800	3	2	--	---		---	--	--	--
Phosphorous												
Total orthophosphate (PO ₄ ⁼)	---		0.08	1	--	--	---		0.00	1	--	--
Silica	4 - 128	17	0.2 - 310	6	2	2	14 - 18		47	1	0	0

Table 4.2.1-6. (Continued)

Water quality parameter	Various oil-shale process waters reported by Fox, et al. (1978)		12 oil-shale process waters analysed during this project			3 Paraho simulated oil-shale leachates	3 UCG condenser waters analysed during this project			
	Range	Average ^b	Range	N ₁ ^c	N ₂ ^c	Range	Range	N ₁ ^c	N ₂ ^c	N ₃ ^c
Solids										
Suspended	---		16 - 100	6	--	---	2.0 - 54	2	--	--
Total	6,350 - 121,000 ^d		576 - 111,000	7	2	---	210 - 1,270	2	2	--
Total dissolved	1,750 - 24,500 ^d	6,800 ^d	3.1 - 111,000	12	6	---	210 - 1,220	2	2	1
Volatile	2,070 - 119,300 ^d		0 - 10,700	7	5	---	0.0 - 50	2	2	--
Sulfur										
Sulfate	42 - 2,200	1,400	3.3 - 17,000	12	5	---	16 - 300	2	1	1
Sulfide	0.0 - 156 ^d		<0.025 - 88	7	0	---	---			

^aValues are mg/l unless otherwise noted.

^bAn average was reported only if values for more than 15 waters were available. Most values were based on 18 waters produced by 4 simulated in situ oil-shale retorts.

^cN₁ = number of waters with values reported; N₂ = number of waters with values outside the range reported by Fox, et al.; N₃ = number of waters with values <0.1 or >10 times the mean value reported by Fox, et al.

^dAppreciable chemical interferences were suspected by Fox, et al.

^e--- = value not reported or analysis not performed.

Some of the values included in these tables were not determined by the procedures recommended in Fox et al. and should be considered only as estimates in lieu of more reliable analyses. From this summary we can generalize about both inter- and intra-processing mode similarities and differences.

All of the 12 oil-shale process waters exhibit at least 1 parameter having a value which deviates from the range reported by Fox et al. Lower values appeared at least once for 27 of the 43 common parameters, while the range was exceeded for 23 parameters. Often both lower and higher deviations of parameters occur in a process water. Thus we may expect future oil-shale water analyses to also display wide variations for chemical parameters.

Although values for the 12 oil-shale effluents vary considerably, these deviations are generally <1 order of magnitude from the means reported by Fox et al. Large deviations are characteristic of only 3 process water samples--LETC ES-78-030 S-54, Paraho Tank-491 and Occidental-6 condenser waters. Whether these deviations can be attributed to analytical problems, oil-shale composition or processing conditions peculiar to that water will be speculated in the following discussion.

Four of the 5 simulated in situ oil-shale retort waters we analyzed were similar to the simulated in situ retort waters reported by Fox et al. (Table 4.2.1-1). Minor deviations for the 2 ES-77-153 drums were low potassium and total dissolved solids, while the ES-78-036 S-55 water displayed low values for ammonia and chemical oxygen demand. No extreme variations appeared in the SOA-75-268 R-13 sample. But the ES-78-030 S-54 water deviated from the means or ranges for 15 parameters. Especially low were sodium, pH and total dissolved solids. Hardness, calcium, chromium, copper, iron, manganese, zinc and sulfate concentrations were >10 times the mean values reported by Fox et al.

To explain the low pH (3.5) of the ES-78-030 S-54 water, we compared the oil-shale compositions of the 5 simulated in situ retorts. While Michigan oil-shale was combusted in the S-54 retort, Colorado oil-shale was retorted to produce the 4 other simulated in situ waters. The Colorado shales contain little sulfur; the Michigan shale assayed at 2 to 3% sulfur, (Dick Martel, LETC, personal communication). Consequently, high sulfate concentrations and low pH in the S-54 water are probably a result of the Michigan shale chemical composition. Retorting probably did not influence these parameters, since the same air-recycle mode was also used in the ES-77-153 retort.

Reinforcing the differences in the chemical composition of the oil shales are the HPLC gradient chromatograms (Figures 4.2.1-1 to 4.2.1-5). Only the ES-78-030 S-54 water contains UV absorbing compounds that elute later than naphthalene, suggesting the presence of many polynuclear aromatic compounds. This compares to a majority of earlier eluting single-ring aromatics and organic acids in the other simulated in situ oil-shale retort waters. Concentrations of phenol in these waters are low.

The chemical analyses and HPLC gradients for the ES-77-153 drum 7 and drum 28 waters suggest a between-barrel uniformity for this retort. Similar homogeneity for 3 Omega-9 water barrels was noted by Fox et al. This suggests that properly handled samples from individual processing modes can be confidently compared in chemical and environmental investigations.

Parameters for the 6 true oil-shale retort waters deviate from the Fox et al. values more often than do the 5 simulated in situ retort waters. And among these waters, the largest number of deviations occur in 13 characteristics of the Paraho Tank-491 sample. Hardness, arsenic and calcium are abnormally high, although ammonia and silica are very low. These values are probably a result of the post-retort handling of this effluent.

Under a thin layer of oil, the Paraho Tank-491 oil-shale retort water samples were contained on-site in a steel tank for almost 3 yr. Although the waters were never heated above 65°C, they may have frozen. Thus chemical, physical and anaerobic biological reactions could have altered the composition of the constituents. Comparison of the Paraho Tank-491 with the Tank-500 values suggests that degradation may have occurred. Both waters were produced from the same shale formation and retorted by the same process, so only the handling and storage of the waters differed appreciably. High arsenic concentration is the only similar parameter. Otherwise alkalinity, chemical oxygen demand, zinc, ammonia, silica and total dissolved solids are much higher in Tank-500. We believe that handling and storage of the Paraho Tank-491 water altered its constitution and invalidated it for use in environmental tests representative of oil-shale retorting.

As noted above, the Paraho Tank-500 oil-shale retort water contained unusually high concentrations of arsenic and zinc. Because such concentrations have not been observed in any other process water, we believe this may be a characteristic of the Paraho oil-shale composition. Also quantified for this water are the highest chemical oxygen demand, ammonia and dissolved solids concentrations observed, although none of these values are >10 times the mean of Fox et al.

For the Geokinetics-9 oil-shale retort water, 11 parameters vary either higher or lower than the ranges reported by Fox et al. Only the sodium and silica concentrations are especially high. Similarly the Omega-9 oil-shale retort water's only extremely high parameter is sodium. These values were probably influenced by the shale compositions of each retort.

Concentrations in the Lawrence Livermore retort, Occidental-6 retort and Occidental-6 condenser waters are either the same order of magnitude or lower than the Fox et al. parameters. Especially low in the Lawrence Livermore sample is iron, while iron, magnesium and ammonia are very low in the Occidental-6 retort water. The ammonia concentration in Occidental-6 condenser water compares well with other waters, but arsenic, iron, magnesium, potassium, zinc, dissolved solids and sulfates are extremely low. This may be characteristic of the processing mode, since vapor condensates seldom contain high salt concentrations.

In the HPLC gradient chromatograms of these oil-shale waters, there is a tendency for the greatest concentrations of UV absorbing compounds to elute early during the gradient, in the region prior to naphthalene. This suggests that these process waters contain predominately single-ring aromatics and organic acids. The only exception to this pattern is the Geokinetics-9 sample, in which a large number of peaks eluting after naphthalene also appear. These suspected polynuclear aromatics are probably associated with the combustion dynamics in the Geokinetics retort. Relatively low concentrations of phenol are indicated in these waters.

When compared to process waters, the simulated leachates of Paraho spent shale contain considerably lower concentrations for almost all of the water quality parameters measured. The pH of these waters is slightly higher (10.4) than for oil-shale and coal gasification waters, but the major cations (sodium, potassium, magnesium and calcium) are all within the normal range of natural waters in the western U.S. And no compound exceeds recommended water quality criteria for single compounds (McKee and Wolf 1963, U.S. EPA 1976, Thurston et al. 1979). Virtually no aromatic organic compounds appear in the HPLC gradient chromatograms (Figure 4.2.1-13). Thus the potential hazards from possible entry of spent oil-shale leachates into receiving waters are substantially less than the hazards posed by the whole process waters.

We cannot generalize about tar sands process wastewaters since we have data for only the Vernal TS-2C composite. While 16 of its chemical parameters exceed the ranges for oil-shale waters, only iron and manganese are unusually high. Conductance, chromium, potassium, sodium, ammonia, pH and sulfates display values lower than the oil-shale ranges. Only the ES-78-030 S-54 simulated in situ oil-shale retort is similar to the tar sands water in terms of the low pH, high iron and high manganese concentrations found in the tar sands water. The low pH of the tar sands wastewater was probably effected by the composition of the combusted substrate. While the tar sands contain low concentrations of sulfur and nitrogen, they also contain little unbound silica and carbonate. Without silicates or carbonates as buffers, the pH of the tar sands wastewater can be readily decreased by organic acids produced during the combustion. Sulfate, though low in concentration, also contributes to the low pH. We feel that chemical composition of the tar sands adequately accounts for the process water constitution, yet different processing modes cannot be discounted without analyzing additional tar sands waters.

The HPLC gradient chromatogram of the Vernal TS-2C wastewater indicates few UV absorbing compounds (Figure 4.2.1-14), with most eluting before the retention time of naphthalene. These compounds are probably single-ring aromatics and organic acids. Little phenol, if any, is present.

With the exceptions of ammonia and phenol, all water quality parameters for the 3 UCG condenser waters are in the same range or lower than the values for simulated in situ oil-shale retort waters reported by Fox et al. Especially low are the major cations (sodium, magnesium, potassium

and calcium) and solids. Such low values reflect that fact that these waters are condensates of vapor streams and, thus, cannot be expected to contain high concentrations of inorganic salts. But the average to high concentrations of ammonia and the extremely high concentrations of suspected phenol appear to be directly attributable to coal combustion. Parkhurst, et al. reported 4,780 mg/l phenol and 1,970 mg/l total ammonia in a hydrocarbonization coal conversion effluent (1979a). For spent still liquors from a plant carbonizing coal in continuous vertical retorts, Herbert reported 4,500 mg/l ammonia and 1,700 mg/l phenol (1962). We feel that this pattern of high ammonia and phenol concentrations in coal processing waters should be further investigated.

Few peaks with retention times greater than naphthalene appeared in the HPLC chromatograms of the coal gasification waters (Figures 4.2.1-15, 4.2.1-16 and 4.2.1-19). Yet the absorbances of the early-eluting peaks, which include the phenolics, are high compared to most oil-shale process waters. The Grand Forks Lurgi process tar-saturated water contains compounds mostly with chromatographic retention times between phenol and naphthalene, although little phenol appears in the gradient chromatogram (Figure 4.2.1-20). Finally, in the retention range between phenol and naphthalene, the Hanna-4B 02W and 03W post-gasification backflood condensate waters display similar, but diluted, absorbances compared to the Hanna-4B 01W condenser water (Figures 4.2.1-17 and 4.2.1-18). This suggests that concentrations of single-ring aromatics can be expected in waters filling old UCG burn cavities.

4.2.1.2 Work in Progress and Planned for Next Year

Chemical characterizations of additional process waters will be completed as the process waters become available.

4.2.2. Toxicity Studies (DeGraeve, Denny, Elder, Geiger, Heath, Hays)

4.2.2.1 Work Completed This Year

During 1979 the following tasks were completed: (1) flow-through 96-hr bioassays exposing fathead minnows (Pimephales promelas) and rainbow trout (Salmo gairdneri) to Paraho oil-shale retort water (1977-1978 composite), Paraho oil-shale retort water (1975-1976 composite), Vernal tar-sands process water (TS-2C composite), Occidental-6 oil-shale condenser water, Occidental-6 oil-shale retort water, Lawrence Livermore Laboratory's Rio Blanco oil-shale retort water and Hoe Creek underground coal gasification (UCG) condenser water, Hanna-4B 01W UCG condenser water, Hanna-4B 02W UCG post-gasification backflood condensate, a phenol plus ammonia mixture, and ammonia alone; (2) flow-through 48-hr bioassays exposing Daphnia pulicaria to all of the above; (3) static acute bioassays exposing juvenile fathead minnows and adult Daphnia pulicaria to Vernal tar-sands process water (TS-2C composite), tar-saturated water (Grand Forks coal gasification, Lurgi Process), a series of Paraho spent oil-shale leachates, Tank-491 Paraho oil-shale retort water (1975-1976 composite), Tank-500 Paraho oil-shale retort water (1977-1978 composite), 5 different LETC simulated in situ oil-shale retort waters, post-gasification groundwaters from Lawrence Livermore Laboratory's Hoe Creek underground coal gasification experimental site and the hydrophilic, acid and basic fractions of Geokinetics-9 and Omega-9 oil-shale retort waters; (4) flow-through embryo-larval bioassays exposing fathead minnow eggs and fry to Occidental-6 oil-shale retort water and Paraho oil-shale retort water (1977-1978 composite); (5) flow-through embryo-larval bioassays exposing rainbow trout eggs and fry to phenol and to Occidental-6 oil-shale retort water; (6) tests determining the avoidance preference response of rainbow trout and fathead minnows to phenol and of rainbow trout to Paraho oil-shale retort water (1977-1978 composite).

Methods Synopsis

Flow-through acute bioassays were conducted according to standard toxicity testing procedures outlined by the U.S. Environmental Protection Agency (1974). Bioassay organisms were exposed to concentrations of test solutions plus well water controls. Rainbow trout and fathead minnows were tested for 96 hr in 28-l and 14-l aquaria respectively, and Daphnia pulicaria were tested for 48 hr in chambers suspended in the 28-l tanks (DeGraeve et al. 1977). In the 96-hr bioassays with phenol plus ammonia, the 2 constituents were mixed and dosed at the same concentrations as found in Hanna-3 condenser water (2,300 mg/l phenol and 21,000 mg/l ammonia as NH_4^+). For static bioassays, fathead minnow larvae and Daphnia pulicaria were exposed for 24 hr in 150 ml beakers.

Embryo-larval bioassays were conducted according to procedures outlined by McKim (1977); fathead minnow and rainbow trout eggs and fry were exposed to 7 toxicant concentrations plus well water controls. Fathead minnow and rainbow trout fry were exposed through 30 and 60 days of age, respectively, at which time fry were individually weighed and measured. Statistically significant reductions in egg hatchability, fry survival

and fry growth were determined by comparing each exposure concentration to controls using Fisher's least significant differences procedure (Neter and Wasserman 1974).

Water quality of test waters during all of the acute and embryo-larval bioassays was determined according to standard procedures as outlined by the American Public Health Association (1975).

For toxicant avoidance tests, a diluter similar to that described by Mount and Brungs (1969) was modified to deliver 4 concentrations of toxicant plus 4 streams of well water to each of 4 avoidance chambers. Each chamber received a continuous well-water flow on 1 side and a continuous toxicant flow on the second side (Figure 4.2.2-1). The 4 toxicant concentrations delivered by the diluter were 100%, 75%, 50% and 25% of the head tank concentration. To initiate the acclimation period 1 fish was placed in each chamber. An overhead movie camera was adjusted to expose 1 frame every 10 minutes, and a curtain was drawn around the tanks to block out any exterior disturbances. During this acclimation period the toxicant head tank contained only well water, so that both sides of the avoidance chamber had identical water conditions. After 24 hr, the toxicant supply pump was activated, which delivered the toxicant to the head tank at a predetermined rate. The head tank was also spiked with the necessary amount of toxicant to bring its concentration immediately up to the desired test level. After the initial 48 hr test period the toxicant and dilution water sides in the avoidance chambers were switched without disturbing the fish, and the test was continued for another 48 hr. At the end of each test all fish were sacrificed, weighed and measured.

Results

Water quality parameters for the test waters are summarized in Table 4.2.2-1 and the results of all flow-through bioassays are presented in Table 4.2.2-2. The toxicities of the 5 oil-shale process waters were variable, with LC_{50} values ranging from 0.068% for rainbow trout exposed to Paraho retort water (1977-1978 composite) to 3.0% for Daphnia pulicaria exposed to Paraho retort water (1975-1976 composite). The 2 UCG condenser waters exhibited similar toxicities, but the Hanna-4B 02W post-gasification backflood condenser water was less toxic than either condenser water collected during gasification. The TS-2C composite of Vernal tar-sands process water was the least toxic of the process waters tested to date.

Un-ionized ammonia was most toxic to rainbow trout; fathead minnows were less sensitive than Daphnia pulicaria. The ammonia plus phenol mixture dosed at concentrations found in Hanna-3 condenser water resulted in LC_{50} dilution percentages similar to those for Hanna-3 water (0.1% for rainbow trout, 0.11% for fathead minnows and 0.18% for Daphnia pulicaria).

In static bioassays (Table 4.2.2-3) leachates from spent oil-shale were not toxic at any concentration to fathead minnows or Daphnia pulicaria. The toxicities of the LETC simulated in situ oil-shale retort waters had similar toxicities, and Daphnia pulicaria were less sensitive to those retort waters than were fathead minnow fry. Post-gasification groundwaters from the Hoe Creek underground coal gasification site were only moderately

Figure 4.2.2-1. Schematic diagram of the modified diluter and test chambers used in five-day continuous flow avoidance studies.

Table 4.2.2-1. Means (ranges) of water quality of test waters during bioassays for process waters and individual constituents.

Water quality parameter	Acute toxicity bioassays		Embryo-larval bioassays	
	Control tanks	Highest concentration toxicant tanks	Control tanks	Highest concentration toxicant tanks
Dissolved oxygen (mg/l)	7.5 (4.9-8.6)	6.9 (3.6-8.3)	6.0 (3.1-8.2)	5.0 (2.7-7.2)
Dissolved oxygen (% saturation)	93 (60-109)	88 (43-109)	86 (47-111)	70 (41-98)
pH (units)	7.9 (7.4-8.3)	7.8 (7.2-8.4)	7.9 (7.6-8.1)	7.9 (7.5-8.1)
Alkalinity (mg/l as CaCO ₃)	149 (138-145)	159 (133-204)	152 (143-159)	160 (148-179)
Hardness (mg/l as CaCO ₃)	572 (510-620)	567 (499-611)	587 (510-649)	597 (512-780)
Conductivity (µmhos/cm at 25°C)	1012 (915-1085)	1117 (905-2000)	1017 (895-1125)	1057 (925-1320)
Free CO ₂ (mg/l)	4.8 (2.1-14.9)	7.5 (1.9-23.5)	5.2 (2.7-10.3)	5.7 (3.1-13.1)

Table 4.2.2-2. Summary of acute, flow-through bioassay results for rainbow trout (Salmo gairdneri), fathead minnows (Pimephales promelas) and Daphnia pulicaria exposed to process water and constituents in 1979.^a

Process water or constituent	Species	Number of organisms per concentration	Highest test concentration	LC ₅₀	Observations
<u>Oil-shale Process Waters</u>					
Paraho retort Tank-500 (1977-1978 composite)	fathead minnows	20	0.4%	0.071%	Lethargic at 0.05%.
	rainbow trout	20	0.4%	0.068%	Lethargic at 0.05%.
	<u>Daphnia pulicaria</u>	10	0.4%	0.147%	48 hr test.
Paraho retort Tank-491 (1975-1976 composite)	fathead minnows	20	3.0%	0.64%	No signs of stress at sublethal concentrations.
	rainbow trout	20	3.0%	0.52%	Rapid operculum at 0.375%.
	<u>Daphnia pulicaria</u>	10	3.0%	3.0%	48 hr test.
Lawrence Livermore retort	fathead minnows	20	0.5%	0.173%	Lethargic at 0.125%.
	rainbow trout	20	0.5%	0.088%	No signs of stress at sublethal concentrations.
	<u>Daphnia pulicaria</u>	10	0.5%	0.153%	48 hr test.
Occidental-6 condenser	fathead minnows	20	1.0%	0.193%	Lethargic at 0.125%
	rainbow trout	20	1.0%	0.176%	Sensitive to disturbance at 0.125%.
	<u>Daphnia pulicaria</u>	10	1.0%	0.193%	48 hr test.
Occidental-6 retort	fathead minnows	20	1.0%	0.65%	Lethargic at 0.5%.
	rainbow trout	20	1.0%	0.35%	Hyperactive at 0.25%
	<u>Daphnia pulicaria</u>	10	1.0%	0.89%	48 hr test.
<u>Tar-sands Process Waters</u>					
Vernal (TS-2C composite)	fathead minnows	20	3.0%	>3.0%	Lethargic at 3.0%.
	rainbow trout	20	3.0%	1.5%	Hyperactive at 0.75%.
	<u>Daphnia pulicaria</u>	10	3.0%	>3.0%	48 hr test.

Table 4.2.2-2. (Continued)

Process water or constituent	Species	Number of organisms per concentration	Highest test concentration	LC ₅₀	Observations
<u>UCG Process Waters</u>					
Lawrence Livermore Hoe Creek condenser water	fathead minnows	20	0.5%	0.21%	No signs of stress at sublethal concentrations.
	rainbow trout	20	0.5%	0.082%	Lethargic at 0.06%.
	<u>Daphnia pulicaria</u>	10	0.5%	--	Inconclusive results.
Hanna-4B 01W condenser water	fathead minnows	20	0.5%	0.33%	No signs of stress at sublethal concentrations.
	rainbow trout	20	0.5%	0.083%	Sensitive to disturbance at 0.06%.
	<u>Daphnia pulicaria</u>	10	0.5%	0.37%	48 hr test.
Hanna-4B 02W post- gasification back- flood condenser water	fathead minnows	20	2.0%	1.4%	Loss of equilibrium at 1.0%.
	rainbow trout	20	2.0%	0.68%	Loss of equilibrium at 0.5%.
	<u>Daphnia pulicaria</u>	10	2.0%	>2.0%	48 hr test.
<u>Constituents</u>					
Ammonia (NH ₃)	fathead minnows	20	2.7 mg/l	1.59 mg/l	Lethargic at 1.2 mg/l.
	rainbow trout	20	2.7 mg/l	0.77 mg/l	Lethargic at 0.55 mg/l.
	<u>Daphnia pulicaria</u>	10	2.7 mg/l	1.16 mg/l	48 hr test.
Ammonia plus phenol	fathead minnows	20	c	0.21%	Hyperactive at 0.15%.
	rainbow trout	20	c	0.11%	Hyperactive at 0.075%.
	<u>Daphnia pulicaria</u>	10	c	0.26%	48 hr test.

^a All acute tests were conducted at 14°C for 96 hr with well water dilution, unless otherwise specified.

^b Rainbow trout mean weight 13g and mean length 95mm; fathead minnow mean weight 0.99g and mean length 38mm.

^c Dosed at concentrations of ammonia and phenol identical to a 0.3 percent dilution of Hanna-3 condenser water (6.9 mg/l phenol, 63 mg/l NH₄⁺). 90

Table 4.2.2-3. Summary of 24-hr acute, static bioassay results for fathead minnows (Pimephales promelas) and Daphnia pulicaria exposed to process waters and process water fractions (NA = not available; NT = no toxicity).

Process water or fraction	LC50 values		Total ammonia concentration (mg/l)
	Fathead minnows	<u>Daphnia</u> <u>pulicaria</u>	
Vernal tar sands process water (TS-2C composite)	0.6%	0.87%	NA
Coal gasification tar-saturated water (Grand Forks, Lurgi process)	1.55%	2.85%	NA
Paraho spent oil-shale leachate (Year 1 simulated annual precipitation)	NT	NT	NA
Paraho spent oil-shale leachate (Year 2 simulated annual precipitation)	NT	NT	NA
Paraho spent oil-shale leachate (Year 3 simulated annual precipitation)	NT	NT	NA
Paraho oil-shale retort water (Tank-491: 1975-1976 composite)	0.9%	2.85%	NA
Paraho oil-shale retort water (Tank-500: 1977-1978 composite)	<0.05%	0.079%	NA
<u>LETC aboveground simulated in situ</u> <u>oil-shale retort waters:</u>			
SOA-75-268 R13	0.071%	0.134%	NA
ES-77-153 Drum 7	0.165%	0.285%	NA
ES-77-153 Drum 28	0.10%	0.245%	NA
ES-78-30 S-54	0.20%	0.285%	NA
ES-78-036 S-55	0.48%	0.85%	NA
<u>Hoe Creek post-gasification groundwaters</u> <u>(Lawrence Livermore):</u>			
Well A (within the gasification zone)	6.7%	>15% ^a	NA
Well 6 (outside of the gasification zone in the direction of aquifer flow)	3.0%	>10% ^a	NA

Table 4.2.2-3. (Continued)

Process water or fraction	LC50 values		Total ammonia concentration (mg/l)
	fathead minnows	Daphnia pulicaria	
<u>Oil-shale process water fractions</u>			
<u>Geokinetics-9 Retort:</u>			
1st half of hydrophilic fraction	5.8%	3.3%	NA
2nd half of hydrophilic fraction	6.1%	3.0%	NA
1st half of distilled water flush of hydrophilic fraction	8.9%	6.4%	NA
2nd half of distilled water flush of hydrophilic fraction	NT	NT	NA
1st half of bicarbonate fraction	NT	NT	NA
2nd half of bicarbonate fraction	NT	67.0%	NA
1st half of distilled water flush of bicarbonate fraction	NT	NT	NA
2nd half of distilled water flush of bicarbonate fraction	80.0	NT	NA
1st half of acid fraction	71.5	NT	NA
2nd half of acid fraction	89.0	NT	NA
1st half of distilled water flush of acid fraction	89.0	76.5	NA
2nd half of distilled water flush of acid fraction	92.0	NT	NA
<u>Omega-9:</u>			
1st half of hydrophilic fraction	2.8	2.1	3,300
2nd half of hydrophilic fraction	3.2	2.1	3,620
1st half of distilled water flush of hydrophilic fraction	3.2	2.3	1,450
2nd half of distilled water flush of hydrophilic fraction	69	NT	6.2
1st half of bicarbonate fraction	NT	NT	9.4
2nd half of bicarbonate fraction	NT	89	0.2

Table 4.2.2-3. (Continued)

Process water or fraction	LC50 values		Total ammonia concentration (mg/l)
	fathead minnows	<u>Daphnia</u> <u>pulicaria</u>	
<u>Omega-9 (Continued) :</u>			
1st half of distilled water flush of bicarbonate fraction	NT	NT	<0.1
2nd half of distilled water flush of bicarbonate fraction	74	NT	<0.1
1st half of acid fraction	62.9	NT	1.7
2nd half of acid fraction	83.8	95.5	0.7
1st half of distilled water flush of acid fraction	87.0	NT	0.5
2nd half of distilled water flush of acid fraction	NT	NT	<0.1

^a No toxicity in highest dilution assayed.

toxic to fathead minnow larvae and even less toxic to Daphnia pulicaria. Oil-shale process waters were tested in static tests only to determine ranges to be used in flow-through bioassays.

The hydrophilic fractions of both Geokinetics-9 and Omega-9 were the most toxic fractions tested in static bioassays. The hydrophilylic fraction of Omega-9 retort water contained substantially more ammonia than the acid or basic fractions.

The results of the avoidance/preference bioassays with rainbow trout are presented in Table 4.2.2-4. These results indicate that although rainbow trout will avoid acutely toxic phenol concentrations, they will not avoid phenol levels which cause reproductive effects. Neither results from the phenol avoidance/preference bioassays with fathead minnows nor Paraho Tank-500 oil-shale retort water bioassays with rainbow trout have been statistically analyzed yet.

In embryo-larval bioassays fathead minnow growth was significantly reduced by 0.0075% Occidental-6 oil-shale retort water (Table 4.2-5) and 0.0016% Paraho Tank-500 1977-1978 composite oil-shale retort water (Table 4.2-6). Hatchability and survival were affected by higher concentrations of both process waters.

Rainbow trout growth was significantly reduced by 0.20 mg/l phenol in a 58 day exposure (Table 4.2-7). We were not able to statistically determine significant differences in survival because only 2 tanks of fry were reared in each concentration of phenol.

Interpretation

UCG Process Waters

In Hanna-3 UCG condenser water both ammonia and phenol were important in affecting the toxicity of that process water to rainbow trout and fathead minnows (DeGraeve et al., 1980). When ammonia and phenol were combined at concentrations found in Hanna-3 condenser water and tested in 96-hr flow-through bioassays with rainbow trout, fathead minnows and Daphnia pulicaria, the LC₅₀ dilution percentages were very similar to those observed for Hanna-3 condenser water diluted in well water. These data suggest that ammonia and phenol, in combination, are primarily responsible for the toxicity of this particular UCG condenser water. Herbert (1962) also speculated that the toxicity of effluents from coal distillation (spent still liquor) to rainbow trout was due primarily to ammonia and monohydric phenol content. He tested this hypothesis by exposing rainbow trout to dilutions of (1) the spent still liquor, (2) ammonium chloride solutions with NH₄⁺ concentrations equal to those in spent still liquor, (3) phenol solutions corresponding to the phenol concentrations in the spent still liquor, and (4) mixtures of phenol plus ammonia at concentrations equal to that in spent liquor. He found, as did we, that even though phenol and ammonia were combined at concentrations less than their respective LC₅₀ concentrations, more than half of the fish usually died. Both our results and Herbert's suggest that there is a synergistic reaction between phenol and ammonia which is primarily responsible for the observed toxicity in these complex coal conversion effluents.

Table 4.2.2-4 Percentage of time spent by rainbow trout in phenol solution and well water during 3 separate avoidance/preference tests.

Phenol conc ^a (mg/l)	Acclimation period				Initial exposure period				Final exposure period			
	A	B	C	D	A	B	C	D	A	B	C	D
TEST 1												
Well water	86%	85%	26%	46%	92%	85%	38%	30%	91%	74%	19%	58%
8.1	14%				8%				9%			
6.4		15%				15%				26%		
4.2			74%				62%				81%	
2.2				54%				70%				42%
N ^b	120	150	151	141	271	273	245	257	276	275	237	200
Chi square stat.	61.6**	74.9**	35.3**	0.9	186.8**	136.4**	13.3**	42.9**	185.1**	68.3**	93.7**	5.8*
TEST 2												
Well water	63%	44%	67%	57%	93%	55%	19%	72%	70%	75%	64%	78%
8.1	37%				7%				30%			
6.5		56%				45%				25%		
4.0			33%				81%				36%	
2.3				43%				28%				22%
N	135	134	135	134	274	274	273	236	267	265	267	264
Chi square stat.	9.1**	1.9	15.0**	3.0	206.7**	2.0	102.2**	47.6**	42.9**	68.8**	22.2**	80.7**
TEST 3												
Well water	36%	31%	64%	82%	89%	65%	43%	31%	84%	73%	43%	66%
6.8	64%				11%				16%			
5.8		69%				35%				27%		
3.2			36%				57%				57%	
1.9				18%				69%				34%
N	138	139	137	134	269	185	263	271	278	271	243	284
Chi square stat.	11.6**	17.3**	10.0**	55.2**	175.1**	16.4**	5.8*	39.2**	127.1**	55.8**	4.5	27.3**

^aPhenol exposure was not begun until the initial exposure period; during the acclimation period both sides of each avoidance tank received well water.

^bNumber of observations for each test period.

* $P \leq 0.05$.

** $P \leq 0.01$.

Table 4.2.2-5. Summary of hatchability, survival and growth of fathead minnow (Pimephales promelas) eggs and fry exposed to Occidental-6 oil-shale retort water.

Concentration (percent)	Un-ionized NH ₃ (mg/l)	Hatchability (percent)	Survival at 30 days (percent)	Mean length at 30 days (mm)	Mean weight at 30 days (g)
0 (Control)	0.006	90.0	79	23.9	0.133
0.0075	0.007	94.5	77	22.2 ^a	0.115 ^a
0.015	0.011	92.0	67	22.9 ^b	0.124 ^b
0.031	0.018	87.6	49	20.9 ^a	0.099 ^a
0.062	0.047	82.8	63	15.9 ^a	0.055 ^a
0.125	0.11	48.0 ^a	25 ^a	11.1 ^a	0.017 ^a
0.25	0.28	3.5 ^a	0 ^a	--	--
0.5	0.82	0.0 ^a	0 ^a	--	--

^aSignificantly reduced relative to control-reared fry ($\alpha = 0.01$).

^bSignificantly reduced relative to control-reared fry ($\alpha = 0.05$).

Table 4.2.2-6. Summary of hatchability, survival and growth of fathead minnow (Pimephales promelas) eggs and fry exposed to Paraho oil-shale retort water (1977-1978).

Concentration (percent)	Un-ionized NH ₃ (mg/l)	Hatchability (percent)	Survival at 30 days (percent)	Mean length at 30 days (mm)	Mean weight at at 30 days (g)
0 (Control)	0.01	90.5	81.7	26.6	0.20
0.0016	0.05	85.1	80.8	26.0 ^a	0.17 ^a
0.0031	0.08	89.1	63.0	26.7	0.16 ^a
0.0063	0.16	69.3 ^a	57.5 ^a	24.8 ^a	0.14 ^a
0.0125	0.30	71.8 ^a	55.8 ^a	17.9 ^a	0.06 ^a
0.025	0.69	46.2 ^a	16.7 ^a	8.6 ^a	0.01 ^a
0.05	1.52	20.0 ^a	---	---	---
0.1	3.35	0.3 ^a	---	---	---

^aSignificantly different ($\alpha = 0.05$) from well-water controls.

Table 4.2.2-7. Summary of survival and growth of rainbow trout (Salmo gairdneri) fry exposed to phenol and well water.

Phenol (mg/l)	Survival at 58 days (%)	Mean length at 58 days (cm)	Mean weight at 58 days (g)
13.8	0	---	---
5.9	106	3.8 ^a	0.46 ^a
2.8	66	4.7 ^a	0.91 ^a
1.1	131	4.7 ^a	0.96 ^a
0.54	186	5.1 ^a	1.18 ^a
0.34	177	5.3 ^a	1.31 ^a
0.20	119	5.4 ^a	1.40 ^a
0.00	181	5.6	1.57
(Control)			

^aSignificantly different ($\alpha = .01$) from well water controls.

Lawrence Livermore's Hoe Creek condenser water and Hanna-4B 01W condenser water had very similar toxic effects on fathead minnows and rainbow trout. Both condenser waters also have high levels of ammonia and phenol (830 mg/l phenol and 4,800 mg/l total ammonia for Hoe Creek, 2,300 mg/l phenol and 3,900 mg/l total ammonia for Hanna-4B 01W). On the other hand, Hanna-4B 02W post-gasification condensate water was considerably less toxic than either condenser water collected during gasification. Ammonia and phenol concentrations were correspondingly reduced in the Hanna-4B 02W sample (200 mg/l phenol and 360 mg/l ammonia). Interestingly, the total ammonia concentration in the Hanna-4B 02W sample was roughly 10% of that amount found in the Hoe Creek and Hanna-4B 01W condenser waters, and the toxicity of Hanna-4B 02W is approximately 10% of that produced by Hoe Creek or Hanna-4B 01W condenser waters. The amounts of phenol in Hanna-4B 02W is also about 10% of that found in Hanna-4B 01W. We conclude from these data that both ammonia and phenol play an important role in the toxicity of these process waters, as they did for Hanna-3 coal gasification condenser water (DeGraeve et al. 1980).

Oil-Shale Process Waters

Unlike UCG condenser waters where high ammonia and phenol concentrations are important toxic constituents in bioassays with rainbow trout and fathead minnows, oil-shale process waters are high only in ammonia while phenol concentrations are considerably lower (see Section 4.2.1). A comparison between toxicity and total ammonia levels for the 5 oil-shale process waters suggests that ammonia is a major factor influencing the toxicity of these waters. The process waters organized in order of decreasing toxicity are: Paraho retort (1977-1978 composite), Lawrence Livermore retort, Occidental-6 condenser, Occidental-6 retort, Paraho retort (1975-1976 composite); the corresponding total ammonia concentrations for these waters are 31.0 g/l, 11.0 g/l, 9.3 g/l, 1.0 g/l and 0.2 g/l. Obviously, the ammonia concentrations and toxicities are directly correlated, and we feel that removal of ammonia from oil-shale process waters would substantially reduce the potential toxicity of these effluents to aquatic biota.

Tar-sands Process Water

The tar-sands water we tested had generally low levels of organic constituents and the total ammonia concentration was only 53 mg/l (see Section 4.2.1). The comparatively low levels of these toxic constituents probably explain why this process water was the least toxic tested to date.

Static Tests

In static tests, the leachates from Paraho spent shale were non-toxic to both fathead minnow larvae and Daphnia pulicaria. Of course these results do not imply that these leachates would not be harmful to aquatic biota in long-term exposures. The high pH values for those leachates (10.4) are 1.4 pH units higher than the pH recommended as maximum by the U.S. EPA (1976) and could have an effect in longer-term exposures. However, the pH effect following discharge into natural receiving waters is likely to be minimal due to low silicate and carbonate buffering intensities of the leachates. The pH of receiving waters is not likely to be altered appreciably and the EPA criterion may not be exceeded.

Although post-gasification groundwaters from the Hoe Creek site were not extremely toxic, our results suggest that in situ gasification of coal could affect groundwater quality. Unfortunately, we have no pre-gasification groundwater toxicity data to compare with. But if we assume that the ambient groundwater was non-toxic, then gasification did decrease the water quality. This potential water quality deterioration would be magnified when large quantities of coal are gasified.

The oil-shale process water fraction data support our belief that ammonia is the principal toxicant in oil-shale retort waters. The hydrophilic fraction is the principal ammonia-containing fraction, as the Omega-9 data indicate (Table 4.2.2-3); this fraction is easily the most toxic in both Omega-9 and Geokinetics-9 fractions.

Embryo-larval Bioassays

The results of a fathead minnow embryo-larval exposure to phenol, presented in the 1 January - 31 December 1978 Annual Progress Report, are again presented in Table 4.2.2-8 for comparison with the results of the embryo-larval bioassay exposing rainbow trout to phenol (Table 4.2.2-7). Both tests produced similar results when the phenol 96-hr LC_{50} values for these species are considered. The lowest phenol concentration reducing growth in the fathead minnow bioassay was 2.5 mg/l, and the highest level producing no measurable effect was 0.75 mg/l. Thus, the long-term no-effect concentration was 1.1% of the 96-hr LC_{50} . Rainbow trout growth was reduced by our lowest phenol exposure concentration (0.20 mg/l), which eliminates the possibility of our finding a no-effect concentration. However, if we assume that a 0.1 mg/l phenol exposure would not significantly reduce growth, the long-term no-effect concentration again is 1.1% of the 96-hr LC_{50} . Therefore, although fathead minnows are less sensitive than rainbow trout in acute bioassays, the relationship of the long-term effect concentration to the LC_{50} is identical to that found for rainbow trout, supporting the contention of Mount and Stephan (1967) that the relationship between acute and long-term toxicity may be consistent among species for a given compound. Our relationship of long-term no-effect concentrations to the 96-hr LC_{50} is slightly lower than the 2.2% and 5.3% which Mount and Stephan (1967) proposed as application factors for fathead minnows exposed to malathion and the butoxyethanol ester of 2,4-D. However, they compared static acute bioassay results to flow-through long-term test results using fathead minnows for both compounds, while we compared flow-through acute tests with flow-through long-term exposures. The potentially different results between static and flow-through acute tests could partially explain the difference in the relationship between long-term and short-term tests. In a review of sublethal effects and "safe" concentrations, Sprague (1971) observed that application factors generally ranged between 0.1 to 0.01, which suggests that the relationship we observed between long-term and short-term effect concentrations is in keeping with the findings of other researchers for other compounds.

Although there are no data in the literature reporting results of phenol embryo-larval bioassays with either fathead minnows or rainbow trout, there are several reports of long-term exposure of other species of fish to phenol. Mikriakov (1969) exposed carp for two 2 mo to 12.5 mg/l

phenol and observed a loss of weight, and Stepanov and Flerov (1969) observed delayed reproduction but no reduced growth in guppies exposed for a year to 12.5 mg/l phenol. Brown and Shurben (personal communication cited by the European Inland Fisheries Advisory Commission, 1973) reported a 20% reduction in growth of rainbow trout exposed for 18 weeks to 1 mg/l of phenol. But the age of the trout was not given, and we assume that they were not newly-hatched fry. Therefore, our results, although they show lower growth effect concentrations for phenol than previously reported, appear to accurately reflect the phenol concentrations which could be expected to affect the most sensitive stage in the life of fish.

Of the oil-shale retort waters tested to date, the Paraho (1977-1978 composite) was the most toxic, reducing fry growth by concentrations as low as 0.0016%. At that dilution the un-ionized ammonia concentration was 0.05 mg/l. Smith and Piper (1975) found reduced growth in rainbow trout exposed to un-ionized ammonia concentrations as low as 0.017 mg/l, or about one-third of the amount found at the 0.0016% dilution. Although they used rainbow trout and we exposed fathead minnows, we feel safe in assuming that enough un-ionized ammonia was present to reduce fathead minnow growth.

In a previous embryo-larval test exposing rainbow trout eggs and fry to Omega-9 retort water (Table 4.2.2-9), we found the growth-effects threshold dilution to be 0.16%. The un-ionized ammonia concentration at that dilution was 0.04 mg/l, which is close to the 0.05 mg/l found to reduce fathead minnow growth in Paraho retort water (1977-1978 composite).

Occidental-6 oil-shale retort water reduced fathead minnow growth at concentrations as low as 0.0075%, and at that dilution only 0.007 mg/l of un-ionized ammonia was present--not enough alone to explain the reduced growth. Apparently some other constituent(s) in this process water may contribute to the reduced fathead minnow growth.

Avoidance Tests

As Table 4.2.2-4 indicates, rainbow trout avoided phenol concentrations ranging from 5.3 to 8.1 mg/l, but did not routinely avoid 1.9 to 4.2 mg/l phenol. These data suggest that the threshold avoidance level for rainbow trout exposed to phenol lies somewhere in the 3.2 to 6.5 mg/l phenol range. We previously determined the 96-hr flow-through LC₅₀ for rainbow trout to be 8.9 mg/l phenol. Since the 5.3 to 6.5 mg/l phenol range was the lowest producing a significant avoidance response, the threshold avoidance level was somewhere between 59% and 73% of the 96-hr LC₅₀ value for rainbow trout. Since we have also observed reduced growth in rainbow trout fry exposed for 60 days to 0.20 mg/l phenol (2.2% of the LC₅₀) it is clear that the avoidance tests were not nearly as sensitive as embryo-larval bioassays for predicting threshold effects.

Our results compare favorably with the findings of other researchers. Jones (1951) reported that the minnow (Phoxinus phoxinus) did not avoid from 4 to 400 mg/l phenol in an avoidance tube. Using the same test species, Stott and Buckley (1979) found that although 10 mg/l phenol killed some fish, concentrations as high as 10 mg/l did not elicit an avoidance response. Sprague and Drury (1969) exposed rainbow trout to 5 concentrations of phenol ranging from 0.001 mg/l to 10.0 mg/l in avoidance tanks and also found no significant avoidance reactions at these levels.

Table 4.2.2-8. Summary of hatchability, survival and growth of fathead minnow (*Pimephales promelas*) fry exposed to phenol and well water.

Phenol (mg/l)	Hatchability (%)	Survival at 30 days (%)	Mean length at 30 days (mm)	Mean weight at 30 days (g)
68.5	45.2 ^a	0	---	---
33.2	77.8	0	---	---
14.5	72.3	26	20.3 ^b	0.18 ^a
6.1	77.2	48	22.1	0.15 ^a
2.5	78.2	50	21.1	0.19 ^a
0.75	81.4	44	22.7	0.25
0.23	82.7	31	22.8	0.18
0.00	81.8	52	22.9	0.27
(Control)				

^aSignificantly different ($\alpha = .05$) from well-water controls.

^bSignificantly different ($\alpha = .01$) from well-water controls.

Table 4.2.2-9. Summary of survival and growth of rainbow trout (Salmo gairdneri) fry exposed to Omega-9 oil-shale retort water and well water.

Omega-9 dilution ^a (%)	Un-ionized NH ₃ (mg/l)	Egg survival (%)	Fry survival (%)	Fry length (mm)	Fry weight ^c (mg)
0 (control)	0.00	92	90	34.3 ± 0.4	360
0.04	0.01	98	98	35.2 ± 0.4	411
0.08	0.02	96	94	33.5 ± 0.2	363
0.16	0.04	90	90	29.3 ± 0.4 ^d	244
0.26	0.07	94	86	24.5 ± 0.3 ^d	142
0.54	0.14	0	0	-	-

^a Percent dilution from calculations based on diluter calibration.

^b Mean ± standard error.

^c Mean based on total weight of all fry in tank.

^d Significantly different (P < .05) from controls.

Our results suggest a somewhat lower avoidance threshold level, which could be related to the test duration. Sprague and Drury only tested their fish for 10 min, Jones exposed his for 4 hr, and Stott and Buckley conducted their tests for a maximum of 12.5 hr. Our longer exposure times (96 hr) may make avoidance tests more sensitive. It seems reasonable that if fish are exposed for longer periods of time, they are more likely to be familiar with their environment and avoid lower concentrations of pollutants.

4.2.2.2 Work in Progress and Planned for Next Year

We are completing an embryo-larval bioassay exposing rainbow trout eggs and fry to Occidental-6 oil-shale retort water. These results will be compared with results from the fathead minnow embryo-larval test with Occidental-6 retort water to determine the relative sensitivities of these two species to oil-shale process waters.

For calendar year 1980 we will emphasize comparative toxicity of treated and untreated process waters and process water fractions. We will select 8 process waters for flow-through acute toxicity testing with rainbow trout, fathead minnows and Daphnia pulicaria. Three process waters will be fractionated into hydrophilic, hydrophobic, acid, basic and neutral fractions, and the toxicity of those fractions to Daphnia pulicaria and newly-hatched fathead minnows will be determined in static bioassays. In addition, we will determine the embryo-larval effects of 2 fossil fuel process waters on fathead minnow eggs and fry.

4.2.3 Degradation Studies (Marcus, Bergman, Meyer Sanchez)

4.2.3.1 Work Completed This Year

Omega-9 and Geokinetics-9 oil-shale retort water degradation experiments begun in 1978 were completed in 1979. These experiments investigated the abilities of microbial populations from an oil-refinery settling pond to metabolize organic compounds in the 2 process waters. Five persisting chromatographic peaks from the 130-day Omega-9 degradation experiment were collected and sent to R. K. Skogerboe's laboratory at Colorado State University for identification. We are awaiting the results from these analyses. In an attempt to evaluate the abilities of microbes from selected natural environments to metabolize process-water organics, a series of experiments were initiated exposing Geokinetics-9 retort water to microbial populations originating from either of 2 sites on the Laramie River, as well as from the oil-refinery settling pond. Following an environmental chamber malfunction, this study was prematurely terminated. To further pursue the objectives of the Geokinetics-9 study, we completed a set of experiments exposing oil-shale process waters from the Occidental-6 retort and condenser and from the 1977-1978 composite (Tank-500) of the Paraho retort to microbial populations from either of the 2 Laramie River sites or the oil-refinery settling pond. Also, this year we explored variations of the analytical methods used for analyzing degradation samples. Our goal was to identify a method which would increase HPLC column life, increase chromatogram reproducibilities and increase chromatogram resolution.

Methods Synopsis

For the original Omega-9 and Geokinetics-9 oil-shale retort water degradation experiments, 6 experimental treatments of each process water were prepared using 175 ml of basal salts medium (Ralston and Vela 1974; also see our 5 December 1977 to 5 March 1978 Quarterly Progress Report). Additions of refinery settling pond water (see 5 June to 5 September 1978 Quarterly Progress Report), distilled water and process water were varied as indicated in Table 4.2.3-1. Treatments 1 to 5 were prepared in sterile, 250-ml screw-cap flasks and agitated at 100 rpm on a gyratory shaker in constant 4300-lux, white fluorescent light at $\sim 28^{\circ}\text{C}$. As a control, Treatment 6 was prepared in a sterile, brown, screw-cap bottle and refrigerated at 4°C . At 10-day intervals during the experiments, samples were aseptically removed from each flask, filtered through a Waters Associates sample clarification kit and collected in 4-ml Teflon-capped glass vials.

Alterations to the chemical compositions in the flasks were monitored by HPLC gradient analyses conducted under the conditions listed on the respective chromatograms in the results. Chromatograms of each sample were qualitatively analyzed for changes in peak heights, disappearance of peaks and appearance of new peaks. Planimetric integrations of the gradient chromatograms from Treatment 1 (1:10 dilution of process water) and treatment 5 (blank background containing no process water) were conducted to determine the area for each 10-min interval on each chromatogram. Values for the background samples were subtracted from the corresponding area of the 1:10 dilution sample to correct each interval's area for the effects of the microbial inoculum. These corrected area values were then analyzed for changes indicating metabolic activity within groups of compounds present in the process waters. The Omega-9 and Geokinetics-9 degradation experiments were terminated after 180 and 140 days, respectively.

Table 4.2.3-1. Contents of the 6 treatment flasks for each of the degradation experiments using Omega-9 and Geokinetics-9 oil-shale retort waters.

Treatment number	Retort water (ml)	Sinclair inoculum (ml)	Sterile d-H ₂ O (ml)	Basal salts medium (ml)	Total volume (ml)
1	20	5	0	175	200
2	4	5	16	175	200
3	2	5	18	175	200
4	20	0	5	175	200
5	0	5	20	175	200
6	20	0	5	175	200

The second Geokinetics-9 degradation series investigated the responses of this retort water to an expanded range of microbial populations. Cultures from two locations on the Laramie River as well as from the oil-refinery settling pond were inoculated into Teflon-lined screw-cap 250-ml Erlenmeyer flasks with the oil-shale retort water according to the scheme presented in Table 4.2.3-2. Treatments 1 to 4, 6 to 8 and 10 to 12 were placed on a 150 rpm gyratory shaker in an environmental chamber programmed for a 16-hr light/8-hr dark photoperiod at $\sim 24^{\circ}\text{C}$; the remaining flasks were wrapped in foil and kept at 4°C . After 65 days the environmental chamber malfunctioned, heating to $>35^{\circ}\text{C}$. At this time we terminated this degradation experiment.

The final set of degradation experiments completed this year exposed oil-shale process waters from the Occidental-6 condenser and retort and from the Paraho retort 1977-1978 composite (Tank-500) to waters containing indigenous microbial populations from the 2 Laramie River sites and the oil-refinery settling pond. Presented in Table 4.2.3-3 is the experimental scheme. Each of the 24 250-ml Teflon-lined, screw-cap, Erlenmeyer flasks contained 180 ml of the selected environmental water with the microbial population and 20 ml of oil-shale process water or distilled water. These latter flasks served to correct process water degradation gradients for backgrounds provided by inocula waters. One set of flasks with each combination of waters was placed on a shaker at 200 rpm in an environmental chamber programmed as described above; the second half of the flasks were each wrapped with foil and refrigerated at 4°C . After 125 days the flasks in the environmental chamber were removed to the refrigerator. Prior to HPLC gradient analyses for each of the 24 flasks, samples were processed through the sample clarification kit. To evaluate alterations to the chemical compositions of the flask contents in the environmental chamber compared to those in the refrigerator, chromatograms from each pair of flasks were overlaid on a light table and interpretative tracings completed indicating areas on the chromatograms which were changed and areas which were essentially unchanged. Due to the density of peaks during the first ~ 15 min of the chromatograms, the relatively low potential hazard and the relatively high potential degradability for these polar compounds, the tracings for the first 15 min of the chromatograms have been omitted.

Included in the alternative HPLC methods evaluation were different solvent systems, column types and injection volumes. Solvent systems tested included 1-hr linear gradients from 50:50 methanol-water to 100% acetonitrile, from 50:50 methanol-water to 75:25 acetonitrile-water, and from 100% water to 100% acetonitrile on the μ -bondapack-CN column; and isocratic chromatograms using concentrations of methanol ranging from 60 to 100% on the μ -C₁₈ column. In addition, HPLC injections ranging from 50 μl to 2 ml were compared at differing detector sensitivities.

Results

Presented in Figures 4.2.3-1 to 4.2.3-4 are HPLC chromatograms for Treatment-1 samples from the 0-day Omega-9 and Geokinetics-9 oil-shale retort waters as well as from the 130-day Omega-9 and 140-day Geokinetics-9 collections. The 130-day Omega-9 HPLC gradient is presented to show the peaks collected and sent to CSU for identification; the final chromatogram

Table 4.2.3-2. Contents of the 13 treatment flasks for degradation experiments with Geokinetics-9 oil-shale retort water in environmental waters from the upper Laramie River (ULR), the lower Laramie River (LLR) and an oil-refinery settling pond (OSP).

Treatment number	Geokinetics water (ml)	Inoculum source (ml)	d-H ₂ O (ml)	Basal salts medium (ml)
1	20	OSP 5	0	175
2	20	OSP 5	0	175
3	0	OSP 5	20	175
4	20	OSP 0	5	175
5	20	OSP 0	5	175
6	20	ULR 180	0	0
7	0	ULR 200	0	0
8	20	ULR 180	0	0
9	20	ULR 180	0	0
10	20	LLR 180	0	0
11	0	LLR 200	0	0
12	20	LLR 180	0	0
13	20	LLR 180	0	0

Table 4.2.3-3. Contents and treatments of 24 treatment flasks for degradation experiments with oil-shale retort waters from the Occidental-6 retort (OR), Occidental-6 condenser (OC) and the Paraho retort Tank-500 1977-1978 composite (PR) and distilled water (DW) controls in environmental waters from the upper Laramie River (ULR), lower Laramie River (LLR) and an oil-refinery settling pond (OSP).

Treatment number	Process water	Environmental water	Treatment
1	DW	OSP	24°C/light/shaken
2	DW	LLR	24°C/light/shaken
3	DW	ULR	24°C/light/shaken
4	DW	OSP	4°C/dark/static
5	DW	LLR	4°C/dark/static
6	DW	ULR	4°C/dark/static
7	OC	OSP	24°C/light/shaken
8	OC	LLR	24°C/light/shaken
9	OC	ULR	24°C/light/shaken
10	OC	OSP	4°C/dark/static
11	OC	LLR	4°C/dark/static
12	OC	ULR	4°C/dark/static
13	OR	OSP	24°C/light/shaken
14	OR	LLR	24°C/light/shaken
15	OR	ULR	24°C/light/shaken
16	OR	OSP	4°C/dark/static
17	OR	LLR	4°C/dark/static
18	OR	ULR	4°C/dark/static
19	PR	OSP	24°C/light/shaken
20	PR	LLR	24°C/light/shaken
21	PR	ULR	24°C/light/shaken
22	PR	OSP	4°C/dark/static
23	PR	LLR	4°C/dark/static
24	PR	ULR	4°C/dark/static

Figure 4.3.2-1. HPLC gradient chromatogram of 0-day Omega-9 Treatment #1.

Figure 4.3.2-2. HPLC gradient chromatogram of 130-day Omega-9 Treatment #1.
Fractions indicated were collected for chemical characterization.

Figure 4.3.2-3. HPLC gradient chromatogram of 0-day Geokinetics-9 Treatment #1.

μ -C₁₈ reverse-phase
500 μ l injection
60 min linear gradient
0-100% CH₃CN/H₂O
2.0 ml/min
254 nmUV; 0.1 AUFS

Figure. 4.3.2-4. HPLC gradient chromatogram of 140-day Geokinetics-9 Treatment #1.

for this degradation series at 180 days has the same general characteristic as the 130-day chromatogram. HPLC analyses of Treatments 2 and 3 for both process waters indicated similar alterations occurring in the chemical compositions of these retort-water dilutions as occurring at the higher retort water concentrations used for Treatment 1.

Comparison of the initial chromatograms for both retort waters indicates that the Geokinetics water contains more organic compounds at higher concentrations throughout the chromatograms (Figures 4.2.3-1 and 4.2.3-3). This is particularly true of compounds in the lower polarity region of the HPLC gradient (>20-min retention). Despite these differences, the degradation trends of the Geokinetics water inoculated with oil-refinery settling pond microbes are similar to the trend observed for the Omega-9 retort water. With both process waters there appeared general peak reductions throughout the chromatograms and peak migrations from the less polar to more polar regions of the chromatograms. Of the peaks initially present in the chromatograms, many either disappeared or are substantially attenuated in the final chromatograms. As the original peaks decreased in height or disappeared, new peaks often simultaneously appeared in the more polar region of the HPLC gradients.

Planimetric integrations of the 10-min chromatographic intervals did not demonstrate well-defined trends. Fluctuation in the data hampered our assessment of the degradation sequences, but the following was observed:

- 1) Areas under the less polar intervals of the chromatograms decrease with time, but not to the background level.
- 2) Areas under the more polar chromatographic intervals fluctuate, sometimes increasing with time complementary to the decrease in less polar intervals.
- 3) Disappearance of defined peaks is not necessarily coordinated with a decrease of the area for the interval(s) in which those peaks occur.

In the 13-flask degradation experiment using Geokinetics-9 oil-shale retort water and microbial populations from 3 environmental sources, HPLC chromatograms indicated the same general alterations to the chemical composition of the Geokinetics water over time as for the first set of degradation experiments. However, those flasks containing Laramie River water displayed less dramatic patterns of peak disappearance and migration than observed for those flasks containing oil-refinery pond water. Among the 2 Laramie River inocula sources, the inoculum from the downstream site within the City of Laramie resulted in greater chemical alteration of the process water than the inoculum from the montane upstream site. As this degradation experiment was prematurely terminated we present no chromatographic analyses for samples from the experimental treatments.

Because results are still being processed from our last set of experiments exposing 3 different process waters to microbial populations from each of 3 different sources, only chromatograms of the 3 process waters exposed to microbial populations from the oil refinery settling pond are presented in this report. A presentation of the entire experiment will

follow in our Final Report. Figures 4.2.3-5 to 4.2.3-7 compare for each process water (Occidental-6 oil-shale retort and condenser waters and Paraho Tank-500 1977-1978 composite oil-shale retort water) the 125-day chromatograms from treatments in the environmental chamber with those from treatments held in the refrigerator. On the figures, those peaks which are stippled indicate peaks present in refrigerated treatments but not in environmental-chamber treatments; cross-hatched peaks are those present in the environmental-chamber treatments but not in refrigerated treatments; and in unmarked peaks are those present in both treatments. In other words, stippled peaks represent areas where the constituent compounds were degraded or otherwise altered by the environmental-chamber treatment; cross-hatched peaks indicate accumulations of potentially recalcitrant metabolic products or other types of persistent degradation products; and clear peaks represent constituents which are relatively resistant to the environmental-chamber treatments. These characterizations are discussed in the Interpretation section following.

Comparison this year of chromatograms from various alternative solvent systems and HPLC column types indicated that the 100% water to 100% acetonitrile 60-min linear gradients on the μ -C₁₈ HPLC column is the most satisfactory method we have tested to analyze samples from process-water degradation experiments. Our results did indicate that using 50 μ l injections and increased detector sensitivity provided chromatograms of similar resolution to the 0.5 to 2.0 ml injections using less sensitivity, suggesting column life could be extended by using smaller injection volumes.

Interpretation

Before discussing the results from our 3 sets of degradation experiments we need to clarify our use of 3 terms. First, when we characterize a chromatographic peak as having been "degraded" through an experimental treatment, we mean that the peak height is reduced in comparison to the corresponding peak of a chromatogram from an alternative or control treatment. While use of degraded may imply mineralization of the compound--an event which likely occurred to many process-water constituents during our degradation studies--we mean by its use that the peak constituent(s) has been altered from the original structure(s) by biological, chemical or physical processes. Similarly, when we describe a peak as a "degradation product" we mean that as a result of an experimental treatment a peak has appeared which did not appear in its companion control treatment. Again, biological, chemical or physical processes may be involved in forming the peak's constituent(s). Finally, we also describe peaks as "persistent." Such peaks occur in both companion chromatograms. Our use of persistent indicates that the peak has remained essentially unchanged, or has grown as a result of contributions by metabolic products, during our experimental treatment and that the peak's constituent(s) is apparently recalcitrant to degradation by microbial populations as well as by chemical and physical conditions present in the flask for the duration of the exposure. We must emphasize that we are not suggesting that the constituents of the "persistent" peaks are recalcitrant to all microbes and all environmental conditions. This will be further discussed below.

Based on our initial degradation experiment using Omega-9 and Geokinetics-9 oil-shale retort waters, we learned that initial process-water concentrations

Figure 4.2.3-6. Interpretive tracings of gradient chromatograms from 125-day exposures to oil-refinery settling pond microbial populations in Occidental-6 oil-shale retort water (see text for descriptions).

Figure 4.2.3-7. Interpretive tracings of gradient chromatograms from 125-day exposures to oil-refinery settling pond microbial populations in Paraho Tank-500 1977-1978 composite oil-shale retort water (see text for description).

of 10% were suitable dilution levels at which to conduct such degradation experiments. Comparison of chromatograms from the 10%, 2% and 1% dilutions in the flasks indicated there was no detectable inhibition of degradation by any retort-water concentration; the patterns of constituent alteration in the flask at the 3 dilutions were similar. This similarity existed for both process waters despite their different chemical natures (cf. Figures 4.2.3-1 and 4.2.3-3). Further, the higher concentrations of constituents in the 10% flask enabled us to follow the alterations to a greater number of process-water constituents over a greater period of time before our analytical methods were insufficiently sensitive to detect changes occurring in the flasks. Therefore, with subsequent degradation experiments we employed a 10% process-water dilution.

As the microbial inoculum used in our first degradation experiment came from an oil-refinery settling pond, the resultant metabolism of process-water organics was mostly indicative of what may occur in waste treatment systems. The results provided little indication of what may happen if process water is accidentally released or if contaminated groundwater seeps into natural surface waters. To examine the abilities of natural microbial communities to metabolize process-water organics, our last 2 sets of degradation experiments used microbial populations from 2 sites on the Laramie River as well as the oil-refinery settling pond. One sampling site on the river was about 27 miles above Laramie, near Woods Landing, Wyoming, and the second site was within the City of Laramie about 0.5 km below a railroad tie plant. While results from our final experimental set are still undergoing analyses, a few observations can be made on the results from the second set. Since this set was prematurely terminated, we stress that these observations are tentative. However, it appears that results from our final degradation experiments will confirm these observations.

As a result of our degradation studies inoculating oil-shale retort water with microbial populations from various sources, we have an indication that microbial populations in natural surface waters do have the abilities to degrade several organic constituents found in such effluent waters. The data indicate, however, that the degradative abilities possessed by populations from the upstream ranching area are less than that of inocula from the downstream municipal area. Greater degradative abilities of the downstream populations may relate to the longer distance the stream travels in reaching the lower site. Over this distance a greater diversity of microbial organisms with alternative metabolic potentials may have developed and become suspended in the water. Specifically, the greater degradative abilities may relate to the long-term seepage of creosote from the tie plant, upstream from the lower site, inducing a resident microbial population possessing enhanced abilities to metabolize aromatic compounds.

Based on a comparison among the 3 interpretative tracings of chromatograms from our last set of degradation experiments, 3 classes of chromatographic peaks are discernible. Class-I peaks are those which were persistent in all 3 of the oil-shale process waters exposed to the refinery pond microbes. Examples of these peaks occurred on the chromatograms at 14.5, 27.7 and 31.0 min retention time. In addition, at 21.8 min a persistent peak was observed for all 3 process waters; however, in the flask containing Paraho Tank-500 retort water in the environmental chamber, the persistent peak

grew--indicating accumulation of a degradation product. And, at 23.0 min a degradative product accumulated in the Occidental-6 condenser water flask during the environmental chamber exposure. Class-II peaks are those which persisted in some process water treatments but were degraded in other treatments. Such peaks occurred at 18.5 min, where the peaks persisted in the Paraho retort and Occidental condenser waters but the constituent compounds degraded in the Occidental retort water, and at 25.0 min, where the peaks persisted in both the Paraho and Occidental retort waters but disappeared in the Occidental condenser water. A final example of this class occurred at 15.0 min where a peak indicating a degradative product accumulated in the Occidental condenser water, a peak persisted in the Paraho retort water and a peak disappeared in the Occidental retort water. The last class of peaks, Class III, are those for which the constituent compounds degraded during the environmental chamber treatments in all 3 process waters, e.g., the peaks at 25.6 and 30.1 min.

This approach to chromatographic peak classification is a valuable step in screening advanced fossil-fuel process wastewaters for potential environmental hazards. Compounds in Class-I peaks pose the greatest environmental and human health hazards. These compounds persist in the system and may cause adverse long term effects through direct chronic ailments or bioconcentration in ecological food chains leading to adverse effects in higher trophic levels. Examples of environmental contaminants producing effects through both modes are well known. However, compounds cannot be permanently placed in Class I on the bases of a single set of experiments, such as we have presented. Persistence of components for a peak in a single set of experiments indicates either (1) that the appropriate microbial systems or physiochemical conditions for degrading the persistent compounds are not present or developing in the flask during the experimental exposure; (2) that the concentration of the persistent compounds present in the flask is inhibiting the system which would degrade the compounds; or (3) that the persistent compounds have a very long half-life in terms of the degradative systems working on the compounds in the flask. It is possible that, of the persistent compounds remaining, some fall into each of the 3 categories. To determine the reason for persistence, individual compounds identified in persistent peaks can be exposed to microbial inocula from various sources, differing concentrations of the compounds can be tested for degradative potentials, degradative kinetics can be determined, and various physiochemical treatment procedures can be explored. Such an array of experiments should be applied to the compounds collected from the Omega-9 samples during our first set of process-water degradation experiments when the identities of these compounds have been made available by the CSU laboratory. For compounds in effluents from advanced fossil-fuel technologies confirmed as belonging to Class I, GC-MS identification followed by additional screening are required to determine potential long-term and bioaccumulation hazards.

Class-II peaks contain compounds with mixed potential hazards. Essentially all compounds in peaks assigned to this class are placed here temporarily until additional data provide a basis for reassignment to Class I or Class III. The mixed degradative responses of peaks in Class II may be the result of any single factor or combination of several factors. To illustrate, a peak eluting at a specific time may contain 1 or more chemical constituents. When there are 2 constituents present, it is possible that 1 may degrade and 1 may persist during an experimental treatment. After

appropriate experiments and analyses have been completed to confirm the fate of the constituents, 1 may become assigned to Class I and the second to Class III. Alternately, initial assignment to Class II may result from biological factors. For example, the components of the mixed microbial inocula which degrade the specific constituents of a peak may be a very small portion of the total inocula in flasks. It is possible that this portion may not be inoculated in some flasks, causing a Class-II response. Constituents of all process-water peaks assigned to Class II require additional study to determine their true potential environmental and human health hazards.

In general, constituents of peaks assigned to Class III are the least hazardous. Compounds in this class are either degraded to H_2O , CO_2 and associated chemicals or altered to another compound. When the latter event occurs and the resultant compound is a Class-I compound, it may be necessary to reclassify the original Class-III compound to Class I. However, such a reclassification would only be done after appropriate studies have proven that the original Class-III compound can be altered, forming a Class-I compound. Studies of this nature would typically be conducted with Class-I compounds from peaks which appeared as a degradative product during an experimental treatment.

During our degradation studies, the observation of perhaps greatest importance was that process-water organics found throughout the polarity range of the chromatographic gradients were subject to degradation by our experimental treatment. This important fact indicates that there is not a polarity range above which or below which degradation of process-water constituents is prevented. Dagley (1972:1) noted "...it is probably true to say that every organic molecule that is synthesized by living matter may also be degraded, in turn, by microbes." Organic constituents of fossil-fuel effluents have originated from living matter; though, we must emphasize that many of these constituents must have been modified during both the fossilization and technological processing. However, it should also be noted that selective enrichment techniques and optimization of environmental conditions have produced microbial cultures able to metabolize many synthetic organic compounds. Our results, which show metabolic activity throughout the polarity range for process-water organics, were produced by "unselected" microbial cultures at ambient environmental conditions. This suggests that many, perhaps most, of the organic compounds present in process waters can be removed in biological treatment systems under environmental conditions which have been optimized for microbial populations produced by selective enrichment techniques with process water organics.

4.2.3.2 Work in Progress and Planned for Next Year

In accordance with the Year 4 supplement to Task Agreement No. 001 of Contract #DE-AS20-79LC0760, efforts on this subtask under this contract are to be terminated at the end of Year 3.

4.2.4 Bioaccumulation Studies (Meyer, Sanchez, Bergman)

4.2.4.1 Work Completed This Year

Adaptation of exhaustive steam distillation to extract process-water organic constituents potentially bioaccumulated by fish was pursued. To this end, rainbow trout exposed to ^{14}C -labelled phenol, p-cresol, naphthalene or benzo(a)pyrene, representing a wide polarity range, were processed to verify extraction efficiencies. Also, short-term bioaccumulation factors were calculated for these static exposures and for fathead minnows from a 96-hr naphthalene flow-through bioassay.

Methods Synopsis

Groups of rainbow trout weighing 7 to 20 g were statically exposed to sublethal concentrations of ^{14}C -labelled phenol, p-cresol, naphthalene or benzo(a)pyrene for durations up to 48 hr in 11-12°C well water. As a preparation for liquid scintillation counting and tissue extraction, the fishes were then sacrificed, homogenized in a Waring blender and divided into at least six 15-20 g subsamples. The exhaustive steam distillation method, described by Veith and Kiwus (1977) for pesticides and PCB compounds, was adapted for extraction of aromatic and polynuclear aromatic compounds (PNAs) in process waters. Combinations of butyl ether, cyclohexane and n-octanol were tested as potential extract reservoir solvents. Briefly, our procedure involved boiling, for 16 hr in 1% H_2SO_4 , either a known addition of one of the ^{14}C -labelled compounds or tissue from fish previously exposed to one of those compounds. Passing through the organic solvent layer before returning to the boiling flask, the refluxed condensates were thus extracted of the co-distilled aromatics while leaving a majority of the non-volatile tissue components behind in the boiling flasks. After the distillation apparatus cooled, 100 ml of each boiling flask containing the fish homogenate mixture was filtered through Whatman #1 paper. Comparisons of the ^{14}C radioactivities in the filtrates with that of the unfiltered mixture indicated the percentage of ^{14}C remaining in the tissue after boiling.

Subsamples of homogenized tissue as well as aliquots of the boiling flask mixture, extract liquids and exposure waters were prepared for liquid scintillation counting (LSC). NCS (Amersham), hydrogen peroxide and acetic acid for digestive tissue plus appropriate volumes of PPO/POPOP/Toluene/X-100 LSC cocktail were added to the scintillation vials prior to ^{14}C assays on a Beckman LS-100C Scintillation System.

Extraction efficiencies were determined using radioassays of each liquid phase in the steam distillation apparatus. Computed first were ^{14}C inventories and percent recoveries. Then the ratio of ^{14}C present in the extract solvent divided by ^{14}C originally introduced in the boiling flask was calculated as the percent extraction efficiency. As an additional refinement for the ^{14}C -phenol analyses, each liquid sample was HPLC fractionated to collect the pre-phenol elution and the phenol peak. Thus the contributions of the ^{14}C -phenol to the total ^{14}C -radioactivity in each liquid phase were determined by LSC assays of each HPLC fraction.

Finally, fathead minnows surviving a 96-hr naphthalene flow-through bioassay at 25°C were extracted to determine uptake at each exposure concentration. Bioaccumulation factors for this and the ^{14}C exposures were determined from the compound's concentration in the fish (mg/kg or DPM/kg) divided by its concentration in the exposure water (mg/l or DPM/l).

Results

Increasing extraction efficiencies for exhaustive steam distillation of phenol-spiked aqueous solutions were obtained using (1) 50% n-butyl ether/50% cyclohexane, (2) toluene, (3) n-butyl ether, (4) 75% n-butyl ether/20% cyclohexane/5% n-octanol and (5) n-octanol, respectively, as the reservoir solvents (Table 4.2.4-1). Using 75% n-butyl ether/20% cyclohexane/5% n-octanol as the reservoir solvent also provided high extraction efficiencies for aqueous solutions of naphthalene exposed fish. However, using this solvent mix, extraction of benzo(a)pyrene from fish tissue was <1% efficient. In general, exhaustive steam distillation has proven of much greater efficiency in extracting aromatic compounds from fish tissue than has tissue homogenization (Table 4.2.4-1).

Recovery of the initial ^{14}C -aromatics in all of these distillations was >90% (Table 4.2.4-2). In addition, removal of ^{14}C -aromatics from the fish tissue was >95% for the phenol, p-cresol and naphthalene experiments; yet filtration of the boiling flask mixture in the benzo(a)pyrene distillation indicated that <14% of that ^{14}C was boiled out of the tissue.

No pre-phenol elution ^{14}C radioactivity was detected after HPLC fractionation and radioassay of the liquid phases from ^{14}C -phenol-spiked exhaustive steam distillations. And no ^{14}C activity was found in the pre-phenol elution of the extract solvent from ^{14}C -phenol-exposed fish boilings, although the boiling flask ^{14}C included 39% pre-phenol elution activity. This corresponded to 20% of the original ^{14}C in the rainbow trout that was not phenol.

Fathead minnow 96-hr bioaccumulation factors in the 5 naphthalene flow-through exposures ranged from 241 to 377 (Table 4.2.4-3). In Table 4.2.4-4 appear the bioaccumulation factors for the static exposures to the 4 compounds tested. Increased bioconcentration generally followed the decrease in polarity of the compounds from phenol to benzo(a)pyrene. For the phenol and p-cresol experiments, disappearance of ^{14}C from the exposure waters was linear and did not exceed 25% in 16 hr. But almost all of the ^{14}C was taken up in the first 4 to 8 hr of the naphthalene and benzo(a)pyrene exposures and could be accounted for in the fish tissue.

Interpretation

As we discussed in our 1978 Annual Progress Report, solvent homogenization is not a suitable technique for extracting phenolic compounds from fish tissue. Exhaustive steam distillation using either n-octanol or n-butyl ether as the organic solvent provides acceptable extraction efficiencies for phenol, although we have encountered problems chromatographing large volumes of n-octanol. For single compound extractions, n-butyl ether appears to be the most acceptable solvent for steam distillation of phenolics.

Table 4.2.4-1. Comparison of extraction efficiencies by various methods for aromatic compounds.

Sample	Solvent homogenization		Exhaustive steam distillation				
	Tetrahydro- furan w/Na ₂ SO ₄	Chloroform w/Na ₂ SO ₄	Toluene (1% H ₂ SO ₄) ^a	n-octanol (1% H ₂ SO ₄)	n-butyl ether (1% H ₂ SO ₄)	50% n-butyl ether / 50% cyclohexane (1% H ₂ SO ₄)	75% n-butyl ether/ 20% cyclohexane/ 5% n-octanol (1% H ₂ SO ₄)
Phenol or ¹⁴ C-phenol spiked in boiling flask	---	---	44% (4 hr) ^b	80% (4 hr) 88% (≥12 hr)	59% (16 hr)	42% (16 hr)	70% (16 hr)
Naphthalene spiked in boiling flask	---	---	---	---	---	---	99% (16 hr)
¹⁴ C-Benzo(a)pyrene spiked in boiling flask	---	---	---	---	---	---	77% (16 hr)
RBT exposed to ¹⁴ C-phenol for 16 hr	Trace	18% ^c	---	80% (16 hr)	39% (16 hr)	---	---
RBT exposed to ¹⁴ C-p-cresol for 16 hr	---	24%	---	---	50% (16 hr)	---	---
RBT exposed to ¹⁴ C-naphthalene for 16 hr	---	---	---	---	---	---	99% (16 hr)
RBT exposed to ¹⁴ C-benzo(a)pyrene for 48 hr	---	---	---	---	---	---	<1% (16 hr)

^aCompound in parentheses denotes boiling flask medium.^bRefluxing times indicated in parentheses.^cValue revised from 1978 Annual Progress Report.

Table 4.2.4-2. Comparison of unextracted ^{14}C -labelled compounds in rainbow trout tissue after 16-hr exhaustive steam distillation in 1% H_2SO_4 .

Compound	% of total ^{14}C remaining in tissue ^a	% of total ^{14}C remaining in boiling flask	% of initial ^{14}C accounted for after boiling
Phenol	4.8	54	90
p-Cresol	3.9	43	91
Naphthalene	0.3	0.7	106
Benzo(a)pyrene	86	>99	93

^a Determined by filtering on #1 Whatman paper the boiling flask potpourri particulates remaining after exhaustive steam distillation; may also include adsorption of compounds onto filter paper.

Table 4.2.4-3. Bioaccumulation factors for fathead minnows exposed to five concentrations of naphthalene in 96-hr flow-through bioassays at 25°C.

Exposure concentration (mg naphthalene/l)	Tissue concentration (mg naphthalene/Kg)	Bioaccumulation factor (mg/Kg/mg/l)
12.7	4,786	377
7.3	2,200	301
3.2	1,050	328
1.5	362	241
0.79	281	<u>356</u>
		Mean = 321
		S.D. = 53

Table 4.2.4-4. Bioaccumulation factors for rainbow trout exposed to ^{14}C -labelled phenol, p-cresol, naphthalene and benzo(a)pyrene.

Compound	Exposure	Exposure time (hr)	Bioaccumulation factor (mg/Kg/mg/l) ^a
Phenol	Static	16	7
p-Cresol	Static	16	26
Naphthalene	Static	16	182
Benzo(a)pyrene	Static	48	177

^aBased on mean exposure concentration in 11-12°C well water and wet tissue weight (dry weight ≈30% wet weight).

A suitable extraction solvent must possess the following physiochemical properties: (1) high partition coefficient from water for phenol and organics having lower polarities, (2) lower density than water, (3) in solubility in water, (4) high boiling point, (5) low UV absorbance, and (6) compatibility with reverse-phase HPLC. Because of its high affinity for PNAs, we chose cyclohexane as a low polarity solvent modifier in the extraction of complex process water organics. Best for extraction of phenol was a 75% n-butyl ether/20% cyclohexane/5% n-octanol mixture, which we used in subsequent exhaustive steam distillations. Higher concentrations of n-octanol would have provided better extraction, but we avoided these because of potential HPLC problems.

We selected naphthalene and benzo(a)pyrene as our ^{14}C -labelled representative PNAs because of commercial availability. Both compounds are adequately distilled from aqueous solutions, and less than 10% of the original compounds are unaccounted for after boiling. But virtually all of the benzo(a)pyrene in exposed fish is retained in the boiling flask potpourri, contrary to the excellent extraction observed with naphthalene-exposed fish. Boiling fish tissue in 1% H_2SO_4 apparently does not break down fats that can extensively partition benzo(a)pyrene, thus preventing its codistillation with water. To better extract PNAs, an alcoholic KOH solution may be required as the boiling flask medium in our exhaustive steam distillations. Before extraction of fish exposed to complex process waters can be successfully attempted, this problem must be resolved.

Over the range of exposure concentrations tested, the fathead minnow 96-hr bioaccumulation factors are relatively closely grouped about the mean value of 321 (Table 4.2.4-3). This suggests the bioaccumulation of naphthalene in these fish was linear with exposure concentration. Previous studies of bioaccumulation with organic compounds have shown that bioaccumulation is a hyperbolic function of exposure duration. Although the species and exposure temperatures differed, the higher average bioconcentration of naphthalene from the 96-hr flow-through bioassay (Table 4.2.4-3) compared to the 16-hr static exposure (Table 4.2.4-4) also suggest a hyperbolic function of naphthalene uptake with time. Thus determination of equilibrium bioaccumulation factors may require flow-through exposures exceeding 96 hr. However, our short-term bioassays indicate rapid uptake of naphthalene and benzo(a)pyrene in the first 4 to 8 hr of exposure as opposed to a gradual, linear uptake rate of phenol and p-cresol during the entire 16-hr exposure.

4.2.4.2 Work in Progress and Planned for Next Year

In accordance with the Year 4 supplement to Task Agreement No. 001 of Contract #DE-AS20-79LC0760, efforts on this subtask under this contract are to be terminated at the end of Year 3.

4.3 Task 3: Methods Development

4.3.1 Bacterial Bioluminescence Assay (Jones, Lebsack, Anderson)

4.3.1.1 Work Completed This Year

We have modified and improved a bacterial bioluminescence assay for measuring toxicity of process waters and process-water constituents from advanced fossil-fuel conversion technologies. With this assay, we have determined EC₅₀ values (the concentration of toxicant inhibiting bacterial bioluminescence by 50%) for 11 oil-shale process waters, 2 in situ coal gasification groundwater samples, 1 tar-sands process water, fractions from 2 oil-shale retort waters, and a series of inorganic and organic constituents from oil-shale or coal gasification process waters. EC₅₀ values for most of these process waters or constituents were compared with acute bioassay results obtained with fish.

Several changes were made in the bacterial bioluminescence assay procedures to shorten the time necessary to process a sample. We also explored various data transformations to determine the best method for obtaining EC₅₀ values from linear regression analyses.

Methods Synopsis

Our instrument for measuring bacterial light output and all reagents were supplied by Beckman Instruments, Inc., Carlsbad, California. The Microtex TM instrument consists of a cooling block, a photomultiplier tube and a chart recorder.

The bacterium Photobacterium fischerii is a luminescent marine bacterium whose light output will decrease in a concentration dependent manner upon exposure to toxicants. Since the normal environment for the organism is sea water, sodium chloride was added to each initial toxicant solution to make a 2% NaCl concentration. Further toxicant dilutions were also made with 2% NaCl.

The bacterial suspension used in the assay is prepared by adding 1 ml of distilled water to a vial of lyophilized bacteria. This mixture is kept on ice and is used for only 1 hr. New bacterial suspensions are prepared as needed. We prepared a 2% NaCl solution used as a blank and as a diluent for preparing the toxicant dilutions.

To perform the assay, 0.5 ml of the 2% NaCl reagent and 10 μ l of the bacterial suspension are placed in a clean glass vial in the 15°C cooling block. Initially the light output increased; after a few minutes it leveled off, then slowly began to decrease. At this time 0.5 ml of the toxicant dilution is added to the vial with the bacteria, and the decrease in bacterial light output is measured after 5 min.

Initially, EC₅₀'s were determined by a computer program that plotted decreases in luminescence, in probits, against the natural logs of the concentrations. However, since the probit transformation is normally only

for use in systems that give quantal responses, another computer program was developed which plots the $\log \Gamma$ against the $\log$ of the concentration of the toxicant ($\Gamma = \% \text{ inhibition} / 100 - \% \text{ inhibition}$). Blanks are also converted to Γ values and subtracted from the toxicant Γ values. Correlation coefficients for linear regression lines are calculated by the computer program. A correlation coefficient close to 1 indicates that the data can be used with some confidence to determine EC_{50} values.

The absorbance of the Geokinetics and Omega-9 fractions was measured at 210 nm using a Beckman 25 spectrophotometer. The fractions were diluted with distilled water to reduce the absorbance below 0.65A. For comparative purposes, the theoretical absorbance of a 1% solution was calculated.

Results

Table 4.3.1-1 lists the EC_{50} values for inhibition of bacterial luminescence for a number of process waters. Also listed are 96-hr flow-through LC_{50} values for rainbow trout and fathead minnows exposed to the same process waters. Figure 4.3.1-1 shows the EC_{50} values for inhibition of bacterial luminescence plotted against the 96-hr flow-through LC_{50} values for rainbow trout in these process waters. The slope of this line is 0.69 indicating that the rainbow trout were more sensitive to the process waters than were the luminescent bacteria. There is a significant correlation coefficient for this regression line (0.82, $p < 0.05$). The fathead minnow 96-hr LC_{50} and the bacterial EC_{50} values (Table 4.3.1-1) were not significantly correlated, with a correlation coefficient of only 0.41. Table 4.3.1-2 compares the EC_{50} values for inhibition of luminescence with static 24-hr LC_{50} values for fathead minnows in another series of process waters. The regression line of LC_{50} and EC_{50} has a slope of 1.3 indicating that the bacterial system was somewhat more sensitive to the waters than were the fathead minnows in static bioassays. There is a significant correlation between bacterial EC_{50} and static fathead minnow LC_{50} values since the regression line has a correlation coefficient of 0.97 ($p < 0.05$).

We also determined EC_{50} values for individual inorganic constituents of Omega-9 oil-shale retort water (Table 4.3.1-3). These EC_{50} values and the correlation coefficients for their concentration-response curves were determined by 2 different methods. The inorganics generally had higher correlation coefficients with the percent inhibition vs. concentration plots. The only exception to this is sodium bicarbonate which had a higher correlation coefficient with the $\log \Gamma$ plot.

Of the 24 Geokinetics-9 and Omega-9 oil-shale retort water fractions, 12 were toxic enough in the bacterial system to give reliable EC_{50} values (Table 4.3.1-4). The remaining 12 fractions were not sufficiently toxic to determine reliable EC_{50} values at the 50% dilution, maximum possible for the assay. Therefore, Table 4.3.1-4 also presents percent inhibition of bacterial bioluminescence measured in the 50% dilution. Additionally, in Table 4.3.1-4 are the UV absorbances at 210 nm calculated for 1% dilutions of the fractions. Overall, high 210 nm absorbance has some relationship to greater inhibition of bacterial luminescence. The less toxic fractions

Table 4.3.1-1. Comparison of bacterial luminescence with flow-through LC₅₀ values for fish exposed to fossil fuel process waters.

Process water	Luminescence EC ₅₀ (%)	Rainbow trout LC ₅₀ (%)	Fathead minnow LC ₅₀ (%)
<u>Oil-shale process waters</u>			
Omega-9 retort	1.3	0.42	0.57
Paraho Tank-491 retort	0.37	0.52	0.64
Paraho Tank-500 retort	1.27	0.068	0.071
Occidental-6 condenser	0.27	0.18	0.19
Occidnetal-6 retort	0.38	0.35	0.65
Lawrence Livermore retort	0.11	0.09	0.17
<u>Tar-sands process waters</u>			
Vernal TS-2C	1.6	1.5	3.0

Figure 4.3.1-1. Relationship among EC₅₀ values for bacterial bioluminescence and LC₅₀ values for rainbow trout with 8 constituents of fossil-fuel processing effluents.

Table 4.3.1-2. Comparison of bacterial luminescence inhibition with LC₅₀ values for fathead minnows exposed to fossil fuel process waters.

Process waters	Luminescence EC ₅₀ (%)	Fathead minnow LC ₅₀ (%)
<u>Oil-shale retort water</u>		
Paraho Tank-491	0.37	0.90
<u>Simulated in situ oil shale retort water</u>		
LETC SOA-75-268 R-13	0.18	0.071
LETC ES-77-153 Drum 7	0.20	0.17
LETC ES-77-153 Drum 20	0.12	0.10
LETC ES-78-30 S-54	0.21	0.20
LETC ES-78-036 S-55	0.65	0.48
<u>Tar sands water</u>		
Vernal TS-2C	1.6	0.60
<u>Post-gasification ground water</u>		
Hoe Creek Well A	5.3	6.7
Hoe Creek Well 6	2.2	3.0

Table 4.3.1-3. Inhibition of bacterial luminescence by inorganic compounds found in Omega-9 water (5-min EC₅₀ in g/l).

Compound	Calculation method			
	<u>Log T vs. log conc.</u>		<u>Concentration vs. % inhibition</u>	
	EC ₅₀	Correlation coefficient	EC ₅₀	Correlation coefficient
Sodium carbonate	0.050	0.92	0.055	0.96
Sodium tetrathionate	0.6	0.94	0.7	0.99
Ammonium bicarbonate	15	0.98	19	0.99
Sodium fluoride	15	0.99	13	0.99
Magnesium chloride	17	0.98	22	0.98
Potassium chloride	18	0.96	23	0.99
Sodium thiocyanate	21	0.98	21	0.99
Calcium chloride	25	0.99	27	0.98
Sodium chloride	25	0.99	28	0.99
Sodium thiosulfate	36	0.93	34	0.97
Sodium bicarbonate	43	0.91	27	0.89
Sodium sulfate	50	0.89	44	0.90

Table 4.3.1-4. Effects of Geokinetic and Omega-9 oil shale retort water fractions on bacterial bioluminescence (5-min EC_{50} in %), 210 nm absorbance of a 1% concentration and static LC_{50} values (in %) to fathead minnows.

Fraction	Fraction description ^a	Luminescence effect ^b		A ₂₁₀	Static LC ₅₀
		EC ₅₀	% Inhibition at 50% dilution		
<u>Geokinetics-9</u>					
1A31	1st ½ hydrophilic fraction	17		1.32	5.8
1A32	2nd ½ hydrophilic fraction	8.2		1.54	6.1
1B31	1st ½ DW Flush	17		0.900	8.9
1B32	2nd ½ DW Flush	45		0.100	>100
2A31	1st ½ bicarbonate elution	>50	47	0.060	>100
2A32	2nd ½ bicarbonate elution	3.9		0.040	>100
2B31	1st ½ DW Flush	>50	46	0.030	>100
2B32	2nd ½ DW Flush	>50	48	0.024	80
3A31	1st ½ acid elution	7.7		0.160	72
3A32	2nd ½ acid elution	20		0.066	89
3B31	1st ½ DW Flush	>50	48	0.049	89
3B32	2nd ½ DW flush	>50	32	0.005	92
<u>Omega-9</u>					
1A51	1st ½ hydrophilic fraction	7.8		0.290	
1A52	2nd ½ hydrophilic fraction	34		0.704	
1B51	1st ½ DW Flush	47		0.410	
1B52	2nd ½ DW Flush	>50	26	0.037	
2A51	1st ½ bicarbonate elution	>50	15	0.015	
2A52	2nd ½ bicarbonate elution	>50	8.5	0.011	
2B51	1st ½ DW Flush	>50	15	0.013	
2B52	2nd ½ DW Flush	>50	12	0.011	
3A51	1st ½ acid elution	5.6		0.200	
3A52	2nd ½ acid elution	17		0.043	
3B51	1st ½ DW Flush	>50	40	0.011	
3B52	2nd ½ DW Flush	>50	6.1	0.003	

^aIn general the first four fractions contain hydrophilics, the second four fractions contain hydrophobic acids and the last four fractions contain hydrophobic bases; there is, however, overlap among the fractions.

^bAs the maximum concentration of toxicant able to be assayed in this test is 50%, for those fractions where the EC_{50} > 50% the % inhibition of luminescence is presented for the 50% dilution.

had absorbances of 0.003 to 0.060 compared to 0.040 to 1.54 for the more toxic fractions. Also, all Geokinetics fractions had greater absorbances than the corresponding Omega-9 fractions; and 9 of the 12 Geokinetics fractions were more toxic in the bacterial system than the corresponding fractions of Omega-9. However, within a group (e.g., the more toxic Geokinetics fractions) the individual correlation of absorbancy and bacterial EC₅₀ is not high. Absorbance is in better agreement with the LC₅₀ value for fathead minnows.

As shown in Table 4.3.1-5, fathead minnows and rainbow trout were generally more sensitive to the phenolic compounds than were bacteria. The slope of the regression line of luminescence EC₅₀ values plotted against rainbow trout 96-hr flow-through LC₅₀ values is only 0.26 (Figure 4.3.1-2) with a correlation coefficient of only 0.36, indicating that there was not a significant correlation. The regression line of luminescence inhibition EC₅₀ against the 96-hr flow-through LC₅₀ to fathead minnows has a slope of 0.24 but a much higher correlation coefficient of 0.67 which implies a significant correlation ($p < 0.05$) between EC₅₀ and fathead minnow LC₅₀ values. The regression line of rainbow trout LC₅₀ values plotted against fathead minnow LC₅₀ values has a correlation coefficient of 0.65 which implies that the relationship between the LC₅₀ values of the two fish species is no better than the correlation of luminescence EC₅₀ values and fathead minnow LC₅₀ values for phenolics.

Interpretation

Reproducible, concentration-dependent decreases in bacterial luminescence resulted when the bacteria were exposed to Omega-9 oil shale retort water. By plotting the log Γ transformations of luminescence inhibition against the log toxicant concentration essentially linear dose-response relationships were obtained.

Bulich (1977) used a similar assay system for determining the toxicity of a number of compounds, including phenol. He found that the minimum concentration of phenol causing at least a 2% decrease in light output was 5 mg/l, and we calculated that his luminescence EC₅₀ was about 60 mg phenol/l. The EC₅₀ values for phenol from Bulich's study are reasonably close to our luminescence EC₅₀ of 25 mg phenol/l, especially considering that Bulich's tests were conducted in a buffered (pH 7) medium and ours were not.

Bulich also compared the results of static acute toxicity tests with fathead minnows to luminescent bacteria exposed to a chemical plant effluent and found that the bacterial system was somewhat more sensitive (EC₅₀ was 4% for bacteria and LC₅₀ was 25% for fathead minnows). When comparing our static fathead minnow bioassays with luminescent bacterial assays, we observed in 3 of the 9 tests that the bacterial system was more sensitive than the fathead minnow tests.

Our data show that in most cases the short-term LC₅₀ values for fish and EC₅₀ values for luminescent bacteria are similar. For the fossil fuel process waters the luminescence EC₅₀ values were usually within a factor of 2 of the fish LC₅₀ values (Paraho Tank-491 being a notable exception). A comparison of the bacterial and fish results for phenolic compounds

Table 4.3.1-5. Comparison of bacterial luminescence inhibition with flow-through LC₅₀ values for fish exposed to phenolic compounds.

Compound	Luminescence EC ₅₀ (mg/l)	Rainbow trout LC ₅₀ (mg/l)	Fathead minnow LC ₅₀ (mg/l)
Resorcinol	310	>100	100
Catechol	32	8.9	3.5
o-Cresol	32	8.4	18
Phenol	25	8.9	68
Benzonitrile	19	32	64
m-Cresol	8.2	8.9	56
p-Cresol	1.3	8.6	29
Hydroquinone	0.079	0.097	0.044
Benzoquinone	0.0085	0.13	0.045

Figure 4.3.1-2. Relationship among EC₅₀ values for bacterial bioluminescence and LC₅₀ values for rainbow trout with 8 advanced fossil-fuel processing effluents.

indicates somewhat more variability, but the general toxicity trends are similar. Overall, for all process waters and phenolic compounds, the fish toxicity tests were more sensitive in about half of the cases.

This type of toxicity testing system has potential value for semi-continuous monitoring of industrial or municipal effluents. If intermittent fish bioassays are completed for comparative purposes, the luminescent bacterial system could be a valuable tool for routinely monitoring the toxic properties of discharges. Thus, this testing method could be used to supplement, or in some cases, replace routine chemical water quality analyses. Regular use of this testing approach could improve the capability for detecting changes in effluent quality which might endanger aquatic biota. If such a change were detected, plant personnel may be able to divert the toxic effluent to storage facilities for later treatment before eventual release.

4.3.1.2 Work in Progress and Planned for Next Year

In accordance with the Year 4 supplement to Task Agreement No. 001 of Contract #DE-AS20-79LC0760, efforts towards development of this method under this contract are to be terminated at the end of Year 3.

4.4 Task 4: Recommendations (Marcus, Bergman, DeGraeve)

As of the end of the Year-3 funding period we present estimated safe concentration (ESC) values for 6 oil-shale retort waters, 1 oil-shale condenser water, 3 UCG condenser waters, 1 UCG post-gasification backflood water condensate, 1 tar-sands extraction process water, and 13 process-water constituents. For all of these waters we have completed 96-hr flow-through acute toxicity bioassays. And we have completed embryo-larval, limited chronic bioassays with 4 of the process waters (Omega-9, Paraho Tank-500 and Occidental-6 oil-shale retort waters plus Hanna-3 UCG condenser water) and 2 process-water constituents (phenol and naphthalene). The results from these bioassays for the most sensitive test species (unless otherwise noted) are presented in Table 4.4-1 along with our ESC values for these process waters.

We use 2 methods to determine ESC values for process waters. First, for those waters on which we have completed embryo-larval chronic bioassays, the ESC value is placed at the dilution level where no adverse effects were observed during the bioassay. Then, by dividing the ESC value by the LC_{50} value determined for the toxicant, an application factor is calculated. Following this procedure, an application factor of 0.01 was determined for both Paraho Tank-500 and Occidental-6 oil-shale retort waters.

Application factors have traditionally been used to calculate estimated safe concentrations for species exposed to toxicants where only acute toxicity bioassays have been completed. For example to estimate the safe exposure level, the LC_{50} value for a species is multiplied by the application factor determined for the toxicant from acute and chronic bioassays with another species. While there is some question on the validity of the application factor concept (Mount 1977), our bioassays with phenol tend to support the concept (see Section 4.4.2). As such, to estimate safe concentration for process waters on which we have completed only acute bioassays, we are using the 0.01 application factor determined in bioassays with Paraho Tank-500 and Occidental-6 oil shale retort waters. We feel this is a legitimate use of application factors because of the general chemical similarity among process waters (see Section 4.2.1).

To calculate ESC values for process-water constituents bioassayed only in acute tests, we are currently using an application factor of 0.05, based mainly on the EPA Blue Book's recommendations. But the application factor we obtained with phenol-exposed rainbow trout suggests that a lower application factor may be more appropriate. Until further data are developed on application factors for process-water related compounds, we will continue to use an application factor of 0.05 to determine ESC values on single compounds for which we have completed only acute toxicity bioassays.

We emphasize that the recommendations we present for ESC values provide preliminary target criteria on which to base the design of process-water treatment systems for proposed fossil-fuel processing technologies. The ESC value for a compound is based on that compound separately in solution; synergistic and antagonistic relationships with other toxicants are not considered.

Table 4.4-1. Summary of recommendations on maximum acceptable toxicant concentrations (MATC) and estimated safe concentrations (ESC) for process waters and single constituents tested in 1977-1979.

waters and single constituents tested in 1977-1979.									
Process water or constituent	Acute toxicity bioassays		Embryo-larval bioassay				Recommendations		Comments
	Most sensitive species ^a	LC50	Species ^a	Most sensitive measure(s)	Threshold effects concentration	MATC	Application factor ^b	Estimated safe concentration	
<u>Process waters</u>									
<u>Oil shale retort water</u>									
Omega-9	RBT	0.42%	RBT	length	0.16%	0.08%	0.2	0.08%	Based on appl. factor for Paraho-500
Geokinetics -9	RBT	0.46%					0.01	0.005%	
Paraho Tank-491 (1975-76 composite)	RBT	0.52%					0.01	0.005%	Based on appl. factor for Paraho-500
Paraho Tank-500 (1977-78 composite)	RBT, FHM	0.07%	FHM	length, weight	0.0016%	0.001% ^f	0.01	0.001%	Based on appl. factor for Paraho-500
Lawrence Livermore	RBT	0.09%					0.01	0.001%	
Occidental-6	FHM ^C	0.65%	FHM	length, weight	0.0075%	0.004% ^f	0.01	0.004%	
<u>Oil shale condenser water</u>									
Occidental-6	RBT	0.18%					0.01	0.002%	Based on appl. factor for Paraho-500
<u>UCG condenser water</u>									
Harna-3	FHM ^d	0.11%	FHM	survival gill histo.	0.01% 0.005%	0.003%	0.03	0.003%	
Hoe Creek	RBT	0.082%					0.01	0.001%	Based on appl. factor for Paraho-500
Hanna-4B 01W	RBT	0.083%					0.01	0.001%	Based on appl. factor for Paraho-500
<u>UCG Post-gasification backflood condensate</u>									
Hanna-4B 02W	RBT	0.68%					0.01	0.007%	Based on appl. factor for Paraho-500
<u>Tar sands wastewater</u>									
Vernal TS-2C (composite)	RBT	1.5%					0.01	0.015%	Based on appl. factor for Paraho-500

Table 4.4-1. (Continued)

Table 4-4-1: (continued)									
Process water or constituent	Acute toxicity bioassays		Embryo-larval bioassay				Recommendations		Comments
	Most sensitive species ^a	LC ₅₀	Species ^a	Most sensitive measure(s)	Threshold effects concentration	MATC	Application factor ^b	Estimated safe concentration	
<u>Process water constituents</u>									
Ammonia (as NH ₃ ⁺)	RBT	0.49 mg/ℓ					0.05	0.02 mg/ℓ	
Benzene	RBT	5.3 mg/ℓ					0.05	0.3 mg/ℓ	
Phenol	RBT	8.9 mg/ℓ	RBT	length,weight	0.2 mg/ℓ	0.1 mg/ℓ ^f	0.01	0.1 mg/ℓ	
Catechol	FHM	3.5 mg/ℓ					0.05	0.2 mg/ℓ	
Resorcinol	FHM	100 mg/ℓ					0.05	5.0 mg/ℓ	
Hydroquinone	FHM	0.02 mg/ℓ					0.05	0.001 mg/ℓ	
o-Cresol	RBT	8.4 mg/ℓ					0.05	0.4 mg/ℓ	
m-Cresol	RBT	8.9 mg/ℓ					0.05	0.4 mg/ℓ	
p-Cresol	RBT	8.6 mg/ℓ					0.05	0.4 mg/ℓ	
Benzonitrile	RBT	32 mg/ℓ					0.05	1.2 mg/ℓ	
p-benzoquinone	FHM	0.04 mg/ℓ					0.05	0.002 mg/ℓ	
Naphthalene	FHM ^e	8.95 mg/ℓ	FHM	hatchability, length,weight	0.85 mg/ℓ	0.45 mg/ℓ	0.05	0.45 mg/ℓ	
Indene	RBT	8.15 mg/ℓ					0.05	0.4 mg/ℓ	

^a RBT = rainbow trout (*Salmo gairdneri*); FHM = fathead minnow (*Pimephales promelas*).

^b Application factors for Omega-9, Paraho Tank-500, Occidental-6 retort, Hanna-3, phenol and naphthalene all computed from TL₅₀ and MATC; all others 0.05 unless otherwise noted comments (also see text for discussion).

^c Rainbow trout were more sensitive with a 96-hr LC₅₀ of 0.35% retort water, but the fathead minnow value (0.65%) used for computation of MATC.

^d Rainbow trout were more sensitive with a 96-hr LC₅₀ of 0.10% condenser water, but the fathead minnow value (0.11%) used for computation of MATC.

^e Rainbow trout were more sensitive with a 96-hr LC₅₀ of 2.2 mg naphthalene/l, but the fathead minnow value at 25°C (8.95 mg naphthalene/l) used for computation of MATC.

^f MATC value estimated because effects were observed at lowest bioassay test dilution.

5.0 ADDITIONAL ACTIVITIES

5.1 Visitors Received

Thomas Rowe and Robert Van Hook, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, visited the group on January 9 to discuss environmental research being conducted, particularly in reference to coal conversion technologies. They also visited Larry Harrington (Project Management Team member at LETC) regarding the same subject.

Pat Molholt, Associate Library Director, Rensselaer Polytechnic Institute, Troy, New York, visited the project February 6-7 and discussed completion of the bibliography.

F. Herbert Bormann, Yale School of Forestry, New Haven, Connecticut, visited with members of the group February 15 and discussed the directions of energy-development related environmental research.

Charles Philpott, Department of Biology, Rice University, Houston, Texas visited the group 12-13 April and discussed aspects of histopathology particularly as related to fish gills.

James McKim, Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency, worked with the group from mid-June to the end of July to study the mechanisms of toxicant uptake by fish.

Kenneth Biesinger and Len Mueller visited the project on 30 August to review project accomplishments and to discuss future research need.

Robert E. Reinert, Coordinator of Fisheries and Wildlife Programs, University of Georgia, visited with project personnel on 10 September and discussed research approaches for investigating aquatic contaminant problems.

Ben Parkhurst, Oak Ridge National Laboratory, visited our laboratory in early October and discussed his work on potential environmental effects of process waters from solvent refined coal.

Del Nimmo, Ecological Consultants, Inc., Fort Collins, Colorado, visited the project on 11 October and discussed his aquatic toxicity studies conducted while at the EPA's Environmental Research Laboratory in Gulf Breeze, Florida.

G. Fred Lee, Engineering Research Center, Colorado State University, visited with the project staff on 1 November and discussed aspects of chemical contaminants in aquatic ecosystems.

Dave Randall, University of British Columbia, visited with project members on 15 November and he presented a seminar covering various aspects of his fish physiology research.

Theodore Snyder, President of the Sierra Club, toured our laboratory and met with project personnel on 6 December during a LETC/College of Engineering sponsored visit.

5.2 Responses to Inquiries

C. E. Cushing, Ecosystems Department, Pacific Northwest Laboratory, Richland, Washington, telephoned in February to inquire about the kinds of research being conducted and the needs for additional research related to environmental aspects of oil shale development. Bergman discussed our project with him and told him of the other research of which we are aware.

John Bowling of Savannah River Ecology Laboratory called several times between February and March to discuss various items of mutual interest to our project and their work with energy-related organic compounds.

W. G. Tucker, U.S. Environmental Protection Agency, Research Triangle Park, N.C., called 9 April to inquire about our project for an overview paper on environmental aspects of synthetic fuel products. He was sent copies of our latest Annual and Quarterly Progress reports.

Linda Koivuniemi, Office of Toxic Materials Control, Michigan Department of Natural Resources, wrote on 10 July requesting information on fossil-fuel constituents and associated toxicities; she was sent our 1978 Annual Report, our last quarterly report and several manuscripts which are in press or submitted.

C. E. Cushing, Battelle Pacific Northwest Labs, called Bergman on 16 July to request that we examine and comment on his experimental plan to study various physiological and ecological parameters during the controlled introduction of oil shale retort water into a stream in western Colorado; we complied.

Robert Clerman, Mitre Corporation, McLean, VA, called in early July to inquire about the nature of our research and request a pre-publication copy of our bibliography; a copy was sent.

Mary Willis, Science News, Washington, D.C., called on 20 July to request information on our research with aquatic ecosystem effects of synfuels development; she was sent copies of several of our recent progress reports plus several manuscripts which have been submitted.

Lisa Moszynski, Energy Policy Information Institute, Boston, called in late July to request information about two recently submitted manuscripts on the aquatic toxicity of fossil-fuel constituents; the manuscripts were sent to her.

C. E. Cushing, Battelle Pacific Northwest Laboratories, called several times in November to discuss our potential involvement with his proposed studies to evaluate responses of a small Colorado stream to additions of oil-shale retort water. Subsequently, on 27 November Bergman, DeGraeve and Marcus met with Cushing, Rodney Skogerboe and James Ward at Colorado State University to discuss mutual involvement in the project.

5.3 Meetings Attended

American Association for the Advancement of Science Annual Meeting,
3-4 January 1979, Houston, Texas.

-Bergman

Colorado-Wyoming Chapter, American Fisheries Society Annual Meetings,
7-8 March 1979, Laramie, Wyoming.

-Bergman, DeGraeve, Geiger, Marcus

EPA Sponsored Oil Shale Symposium and Paraho Environmental Data
meeting. 26-28 March 1979, Denver, Colorado.

-Bergman, DeGraeve, Lebsack

Joint meeting of the Southwest Division of the American Association
for the Advancement of Science and the Colorado-Wyoming Academy of Science,
25-28 April 1979, Durango, Colorado.

-Bergman, DeGraeve

The Mitigation Symposium - A National Workshop on Mitigating Losses
of Fish and Wildlife Habitats. 16-20 July 1979, Fort Collins, Colorado.

-Marcus

Annual Meeting of the Ecological Society of America. 13-16 August
1979, Stillwater, Oklahoma.

-Bergman, Parker

Annual Meeting of the American Society for Pharmacology and Experi-
mental Therapeutics. 19-23 August 1979, Portland, Oregon.

-Anderson, Lebsack

Workshop on Biotransformation and Fate of Chemicals in the Aquatic
Environment. 19-24 August 1979, Pellston, Michigan.

-Bergman

Annual Meeting of the American Fisheries Society. 12-15 September
1979, West Yellowstone, Montana.

-Bergman, DeGraeve, Geiger, Johnson, Zeveloff

Fourth Annual American Society for Testing and Materials Symposium
on Aquatic Toxicology. 16-17 October 1979, Chicago, Illinois.

-Bergman, DeGraeve

5.4 Presentations

5.4.1 Abstracts Submitted

All abstracts submitted this year have been presented.

5.4.2 Presentations Delivered

"Environmental effects of underground coal gasification with emphasis on aquatic ecosystems." H. L. Bergman and G. M. DeGraeve. Presented at Symposium on Underground Coal Gasification. Am. Assoc. for the Advancement of Science Annual Meetings, 3 January 1979, Houston, TX (copy of abstract presented in 5 June - 5 September 1978 Quarterly Progress Report).

"Potential environmental effects of underground coal and oil shale extraction technologies." G. M. DeGraeve and H. L. Bergman. Presented at Annual Meeting of the Colorado-Wyoming Chapter of the American Fisheries Society, 7 March 1979, Laramie, Wyoming (copy of abstract presented in 15 December 1978 - 15 March 1979 Quarterly Progress Report).

"Aquatic effects of effluents produced from in situ recovery of shale and coal." G. M. DeGraeve and H. L. Bergman. Abstract submitted to the 1979 Joint Meeting of the Southwest Division of the American Association for the Advancement of Science and the Colorado-Wyoming Academy of Science, 25-28 April 1979, Durango, Colorado (copy of abstract presented in 15 December 1978 - 15 March 1979 Quarterly Progress Report).

"Inhibitory interactions of aromatic organics during microbial metabolism." J. S. Meyer, H. L. Bergman and M. D. Marcus. Ecological Society of America Annual Meeting, 12-17 August 1979, Stillwater, Oklahoma (copy of abstract presented in 15 March - 15 June 1979 Quarterly Progress Report).

"The aquatic toxicity of several oil shale process waters: a comparison." G. M. DeGraeve. American Fisheries Society Annual Meeting, 12-15 September 1979, West Yellowstone, Montana (copy of abstract presented in 15 December 1978 - 15 March 1979 Quarterly Progress Report).

"A scanning electron microscopy study of the development of lateral line neuromasts in fathead minnow (Pimephales promelas) larvae." D. L. Geiger and R. D. Johnson. American Fisheries Society Annual Meeting, 12-15 September 1979, West Yellowstone, Montana (copy of abstract presented in 15 December 1978 - 15 March 1979 Quarterly Progress Report).

"Effects of sublethal concentrations of oil shale retort water on heart and opercular rates of unrestrained rainbow trout." F. N. Lamming, M. J. Yakimovich and H. L. Bergman. American Fisheries Society Annual Meeting, 12-15 September 1979, West Yellowstone, Montana (copy of abstract presented in 15 December 1978 - 15 March 1979 Quarterly Progress Report).

"Sublethal effects of an in situ oil shale process water on rainbow trout." A. D. Anderson and M. E. Lebsack. American Society for Pharmacology and Experimental Therapeutics, 19-23 August 1979, Portland, Oregon (copy of abstract presented in 15 June - 15 September 1979 Quarterly Progress Report).

"The use of bacterial luminescence to measure the toxicity of fossil fuel process waters and selected constituents." M. E. Lebsack, A. D. Anderson and G. M. DeGraeve. Fourth Annual American Society for Testing and Materials Symposium on Aquatic Toxicology, 16-17 October 1979, Chicago, Illinois (copy of abstract presented in 15 March - 15 June 1979 Quarterly Progress Report).

5.5 Publications

5.5.1 Papers in Preparation

"Modification of a proportional diluter to study the avoidance response of rainbow trout to phenol." G. M. DeGraeve. (In Review)

"Effects of naphthalene and benzene on fathead minnows and rainbow trout." G. M. DeGraeve, R. L. Elder, D. C. Woods and H. L. Bergman. (In Review)

"Use of a modified factorial design to evaluate environmental interactions on maximum degradation rates." T. E. Fannin, H. L. Bergman, D. A. Anderson and M. D. Marcus. (In Review)

"Comparison of bacterial luminescence and fish bioassay results for fossil-fuel process waters. M. E. Lebsack, A. D. Anderson, G. M. DeGraeve and H. L. Bergman. (In Review)

"Bibliography of aquatic ecosystem effects, analytical methods and treatment technologies for organic compounds in advanced fossil-fuel processing effluents - Volume 2" (Tentative title). L. Zeveloff, M. D. Marcus, M. Parker and H. L. Bergman.

"Extraction of phenol, naphthalene and benzo(a)pyrene from rainbow trout (tentative title) J. S. Meyer, H. L. Bergman and J. Anderson.

"Biodegradation of process waters from oil-shale retorting and coal gasification" (Tentative title). M. D. Marcus, H. L. Bergman and J. S. Meyer.

"Reevaluation of the contribution of ammonia to toxicity of coal conversion effluents (tentative title). J. S. Meyer, G. M. DeGraeve and H. L. Bergman.

5.5.2 Papers Submitted

"Sublethal effects of an in situ oil shale retort water on rainbow trout." M. E. Lebsack, A. Duane Anderson and K. F. Nelson and D. S. Farrier. Submitted to Toxicology and Applied Pharmacology.

5.5.3 Papers in Press

Anderson, A. D., M. E. Lebsack, G. M. DeGraeve, D. S. Farrier and H. L. Bergman. (In Press). Toxicity of an in situ oil shale process water to rainbow trout and fathead minnows. Archives of Environmental Contamination and Toxicology.

Marcus, M. D., H. L. Bergman, D. A. Stoller, S. K. Brown and P. A. Molholt. (In Press). Bibliography of aquatic ecosystem effects, analytical methods and treatment technologies for organic compounds in advanced fossil-fuel processing effluents. Ecological Research Series, U.S. Environmental Protection Agency, Duluth.

DeGraeve, G. M., D. L. Geiger, J. S. Meyer and H. L. Bergman. (In Press). Acute and embryo-larval toxicity of phenolic compounds to aquatic biota. Archives of Environmental Contamination and Toxicology.

DeGraeve, G. M., R. L. Overcast and H. L. Bergman. (In Press). Toxicity of underground coal gasification condenser water and selected constituents to aquatic biota. Archives of Environmental Contamination and Toxicology.

5.5.4 Papers Published

Bergman, H. L., R. M. Carlson, C. W. Gehrs, M. Katz and M. L. Landolt. 1979. Phenol. p. 221-228 in: R. V. Thurston, R. C. Russo, C. M. Fetterolf, T. A. Edsall and Y. M. Barber, Jr. (eds.). Review of EPA Red Book: Quality criteria for water. Water Quality Section, American Fisheries Society, Bethesda, MD.

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