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

By
Harold L. Bergman
A. Duane Anderson

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Department of Zoology and Physiology and
Department of Pharmacology and Biochemistry
University of Wyoming
Laramie, WY 82071

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FUEL PROCESSING TECHNOLOGIES ON AQUATIC SYSTEMS

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Submitted by

Harold L. Bergman
Department of Zoology and Physiology
and
A. Duane Anderson
Department of Pharmacology and Biochemistry

The University of Wyoming
Laramie, Wyoming
82071

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1.0 SUMMARY

This is the first annual progress report on a three-year EPA-DOE, jointly funded project to evaluate the effects of aqueous effluents from in situ fossil fuel processing technologies on aquatic biota. Some of the activities seven months into the project continue to emphasize setting up equipment and facilities and adjusting experimental methods.

Of the five project tasks, we have now completed or are well underway with all work proposed for completion during Year 1, and most of the specific sub-tasks not actually completed will be finished early in Year 2. Accomplishments on the five project tasks during Year 1 (15 May - 15 December, 1977) were as follows:

Task 1: Literature Review

- Completed computer searches for literature on 57 organics known to occur in fossil fuel process waters, with about 1500 citations obtained.
- Evaluated relevance and importance of these citations and abstracts, selecting approximately 500 for which the full papers have now been obtained for further review, analysis and summary.
- Concluded that published information on most of the organics is extremely scarce, with most of the search output including literature on only phenol, cresol, catechol, resorcinol, naphthalene, aniline and biphenyl.
- Completed reviews on nine heavy metals known to occur in advanced fossil fuel process waters.

Task 2: Toxicity Studies

- Completed flow-through, 96-hr toxicity bioassays on seven organic compounds found in coal gasification process water with 96-hr TL₅₀ values in mg/l for rainbow trout/fathead minnows as follows: phenol (8.9/67.5); catechol (8.9/3.5); resorcinol (>100/100); o-cresol (8.3/18.2); m-cresol (8.9/55.9); p-cresol (8.6/28.6); and naphthalene (2.25/4.9).
- Completed static 48-hr or 96-hr toxicity bioassays on Hanna 3 coal gasification condenser water with fathead minnow fry, fathead adults, rainbow trout juveniles and Daphnia pulex; 96-hr TL₅₀ values ranged around 0.1% condenser water in all tests.
- Partially completed a fathead minnow embryo-larval test on Hanna 3 condenser water, with preliminary results showing egg hatchability affected adversely at least down to 0.04% condenser water.

- Completed static and flow-through 96-hr toxicity bioassays of Omega 9 oil shale retort water with rainbow trout and static tests with fathead minnows; the 95% confidence interval for the TL₅₀ on flow-through tests with rainbows (the most sensitive of the two species) was 0.38% to 0.39% Omega 9 water.
- Partially completed a rainbow trout embryo-larval test on Omega 9 oil shale retort water, with preliminary results showing effects on egg hatchability at 0.54% Omega 9 water.
- In toxicant avoidance tests, rainbow trout avoided Omega 9 water at concentrations greater than the TL₅₀.
- Thick-section, light-microscopy investigations of histopathology response to process waters and individual compounds were found to be unsatisfactory as a routine tool because of inadequate sensitivity.

Task 3: Degradation Studies

- Tested several systems for evaluating biological and physical/chemical degradability of organic compounds found in process waters from advanced fossil fuel technologies.
- Obtained preliminary bacterial degradation rate information on phenol, o-cresol, m-cresol and p-cresol, with results all indicating degradation half-times of several hours in our test system.

Task 4: Bioaccumulation Studies

- Determined bioaccumulation factors for phenol in organisms from aquatic microcosm experiments; however, concentration factors were determined on the basis of phenol equivalents, thus the concentration factors obtained represent maximum values. Real concentration factors are small, probably approaching a value of 1.
- Acquired and set up a high performance liquid chromatograph (HPLC) and accessories necessary for organic residue analyses in future bioaccumulation studies.
- Tested several tissue extraction and clean-up procedures for phenolics, concluding that the best methods may entail either steam distillation or homogenization with a relatively polar solvent and clean-up on an HPLC gel permeation column.
- Worked up HPLC methods for quantifying single organic compounds in water and for characterizing organics in coal gasification process water.

Task 5: Recommendations

- Based on literature reviews, estimated safe concentrations for evaluation of potential process water releases to aquatic environments for arsenic (0.005 mg/l); cadmium (0.0001 mg/l in soft water and 0.0012 mg/l in hard water); chromium (0.02 mg/l); cobalt (0.5 mg/l); copper (0.004 mg/l); manganese (0.1 mg/l); nickel (0.03 mg/l); selenium (0.01 mg/l); and zinc (0.001 mg/l in soft water and 0.01 mg/l in hard water).
- Recommended tentative adoption of EPA Red Book values for estimated safe concentrations of ammonia (20 $\mu\text{g/l}$ as NH_3), cyanide (5.0 $\mu\text{g/l}$ as CN^-) and hydrogen sulfide (2.0 $\mu\text{g/l}$ as H_2S).
- Estimated safe concentrations in aquatic ecosystems for seven organics (phenol, catechol, resorcinol, o-cresol, m-cresol, p-cresol and naphthalene) found in coal gasification condenser water (see Table 4.5-2).
- Concluded that all estimated safe concentrations that we report during the project should only be used as preliminary performance criteria for proposed process water treatment systems. Final performance criteria recommendations will be presented in our completion report at the end of Year 3.

Several problems prevented completion of some proposed tasks for Year 1. The principal sub-tasks remaining to be completed, reasons for being unable to complete these tasks, and our projected schedule for completion are as follows.

- Bibliographic searches for all published literature on aquatic ecosystem effects of organic compounds known to be in situ fossil fuel process waters: we decided to expand the search to produce a publishable bibliography; this work and a draft of the completed bibliography should be completed near the end of the first quarter of Year 2.
- Toxicity tests on fractions of underground coal gasification (UCG) process water and fractions of Omega-9 oil shale retort water: sufficient UCG process water was not available from LERC for us to fractionate and the Omega 9 fractions to be provided by LERC were not yet available; all of these tests will be conducted when the process waters and fractions are available from LERC, probably during the first half of Year 2.
- Limited chronic (embryo larval) toxicity tests on one model compound (a phenolic): we were delayed in getting sufficient space and equipment for setting up an additional diluter for these tests until this past fall; this test will be conducted during the first half of Year 2.

-Degradation tests on four to six model compounds, although we do have preliminary degradation rate determinations on four compounds: we were not satisfied with results obtained from Metcalf microcosms for these tests and it was necessary to develop a new bacterial degradation test which we now have worked up; complete results on at least four and possibly six compounds will be available at the end of the first quarter of Year 2.

-Bioaccumulation data on model compounds tested in acute bioassays: we were delayed in obtaining required analytical equipment (a liquid chromatograph) until November; we should catch up with all bioaccumulation tasks during the first half of Year 2.

2.0 INTRODUCTION

This is the first annual progress report issued under Task Agreement #1, DOE-UWRMIEE Contract #EY-77-C-04-3913, a project to evaluate the effects of aqueous effluents from in situ fossil fuel processing technologies on aquatic biota. The three-year project is funded on a one year renewable basis 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 Research Center.

Briefly, the goals of the project are to:

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 1 are being pursued through five tasks:

1. Literature review on process water constituents;
2. Toxicity studies on the effect of process waters and six process water constituents on aquatic biota;
3. Degradation rate studies on four to six process water constituents;
4. Bioaccumulation studies on four to six process water constituents; and
5. 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, the progress toward completion of these goals is presented in sections on Administration, Task Reports, and Additional Activities.

3.0 ADMINISTRATION

3.1 Staff

The following individuals are now working either part-time or full-time on the various project tasks (see Section 4, TASK REPORTS, for task assignments).

- Harold L. Bergman (Co-Principal Investigator and Contact)
- A. Duane Anderson (Co-Principal Investigator)
- Michael Parker (Co-Investigator)
- G. Michael DeGraeve (Research Associate)
- Timothy Fannin (Research Assistant)
- Rodney Johnson (Research Assistant)
- Michael Marcus (Scientist I)
- Joseph Meyer (Scientist I)
- Dennis Woods (Technician)
- Ramona Jones (Technician)
- Carolyn Larusso (Technician)

3.2 Staff Meeting and Seminars

During the first quarter of this project, 5 June to 5 September 1977, several ad hoc staff meetings were held to initiate the project tasks. Starting on 28 October 1977 we began holding regular bi-weekly staff meetings to review progress, present results and conclusions for staff criticism, and plan up-coming work. Each meeting is devoted to one task. To date the following task accomplishments have been presented and discussed:

October 28	Literature Review (Marcus)
November 11	Toxicity-Coal Gasification (DeGraeve) and Oil Shale (Anderson)
November 18	Toxicity - Single Compounds (DeGraeve)
December 9	Degradation (Fannin, Parker)

Staff meetings will continue on the second and fourth Friday each month (except holidays), with the discussion of the tasks rotated as scheduled through the first quarter of 1978:

December 16	Bioaccumulation (Meyer)
January 13	Recommendations (Bergman)
January 27	Literature Review (Marcus)
February 10	Toxicity (DeGraeve)
February 24	Degradation (Fannin, Parker)

On 17 November Dr. John Cairns, Jr., from VPI-SU in Blacksburg, Virginia, visited the project and presented a seminar entitled "Disaster Prediction and Ecosystem Monitoring."

3.3 Administrative Problems

The Task Agreement, Budget and EPA-DOE Interagency Agreement are currently being processed for calendar year 1978 funding. Presumably, necessary approvals will be in hand prior to 1 January 1978 to avoid interruption in work.

4.0 TASK REPORTS

4.1 Task 1: Literature Review (Marcus, Meyer, Bergman)

4.1.1 Work Completed This Year

Methods Synopsis

Seven computerized bibliographies have been searched to provide relevant literature published from 1970 to the present on 57 organic compounds known to occur in process waters from fossil fuel conversion technologies. These searches were conducted through the U.W. Science Library and the Denver Public Library Center for Research and included the bibliographic data bases SSIE, BIOSIS PREVIEWS, POLLUTION, CDI, CHEMCON, NTIS and ENVIROLINE (respectively, Smithsonian Science Information Exchange, Biological Abstracts and BioResearch Index, Pollution Abstracts, Comprehensive Dissertation Index, Chemical Abstracts, National Technical Information Abstracts based on the Government Reports Index and Government Reports Announcements, and ENVIROLINE produced by the Environmental Information Center). Organic compounds emphasized in the searches are presented in Table 4.1-1.

The strategy used in the searches involved crossing each compound and its synonyms with appropriate key words to identify available literature for each compound pertaining to toxicity, degradation, bioaccumulation, cycling and structure-activity. Key words used have been presented in Appendix B of our quarterly report for the period 5 June to 5 September 1977.

The citations obtained from the searches were reviewed to select those of apparent interest to our studies. For those citations provided by BIOSIS PREVIEWS, CHEMCON, POLLUTION and CDI, the abstracts of interesting citations were hand searched in the library. Subsequently, copies of all important papers were obtained and reviewed both for content and for important citations of papers appearing before 1970.

To provide recommendations on estimated safe concentrations in aquatic environments for selected inorganic constituents occurring in process waters from advanced fossil fuel technologies, computerized bibliographic and hand searches have been performed similar to those procedures presented above.

Results

The output from the searches provided over 1500 citations. Of these, approximately 500 papers have been obtained. However, relatively few of all the organic compounds searched are represented in the output. Those compounds emphasized in the output include phenol, cresol, catechol, resorcinol, naphthalene, aniline and biphenyl.

A partial review of the literature concerning phenol was presented as Appendix C of the previously cited quarterly report. In addition, some of the more important short-term toxicity information on phenol is summarized in Table 4.1-2. Analysis and summarization is progressing rapidly for all citations we have obtained relevant to evaluating possible aquatic effects of aqueous effluents from in situ fossil fuel processing technologies.

Table 4.1-1. Organic compounds surveyed on computerized bibliographic searches conducted during 1977.

phenol	dimethyl pyridine
o-cresol	ethyl toluene
m-cresol	benzaldehyde
p-cresol	cyanobenzene
catechol	methyl styrene
resorcinol	1, 2, 4-trimethyl benzene
hydroquinone	1, 2, 3-trimethyl benzene
acetaldehyde	benzofuran
hexadecane	trimethyl thiophene
benzo(a)pyrene	indane
propanenitrile	indene
methyl ethyl ketone	methyl indane
methyl isopropyl ketone	methyl benzofuran
n-butyronitrile	2-ethyl phenol
2-pentanone	3-ethyl phenol
3-pentanone	2, 4-dimethyl phenol
pyrrole	n-dodecane
n-pentane nitrile	dimethyl indane
2-methyl thiophene	naphthalene
cyclopentanone	benzothiophene
methyl pyrrole	4, 7-dimethyl benzofuran
methyl cyclopentane	n-tridecane
methyl pyridine	methyl naphthalene
hexane nitrile	tetradecane
ethyl benzene	biphenyl
octene	ethyl naphthalene
aniline	dimethyl naphthalene
dimethyl thiophene	acenaphthene
	hexadecene

Table 4.1-2. Literature summary of TL₅₀ values for the toxicity of phenol to fish.

Test species	Test	Test length (hours)	Temp (°C)	pH	Alk (mgCaCO ₃ /l)	Hardness (mgCaCO ₃ /l)	TL ₅₀ (mg/l)	Reference
Fathead	static	24	25	7.5	18	20	40.60	Pickering & Henderson 1966
Fathead	static	48	25	7.5	18	20	40.60	Pickering & Henderson 1966
Fathead	static	96	25	7.5	18	20	34.27	Pickering & Henderson 1966
Fathead	static	24	25	8.2	300	360	38.62	Pickering & Henderson 1966
Fathead	static	48	25	8.2	300	360	38.62	Pickering & Henderson 1966
Fathead	static	96	25	8.2	300	360	32.00	Pickering & Henderson 1966
Bluegill	static	24	25	7.5	18	20	25.85	Pickering & Henderson 1966
Bluegill	static	48	25	7.5	18	20	23.88	Pickering & Henderson 1966
Bluegill	static	96	25	7.5	18	20	23.88	Pickering & Henderson 1966
Goldfish	static	24	25	7.5	18	20	49.86	Pickering & Henderson 1966
Goldfish	static	48	25	7.5	18	20	49.13	Pickering & Henderson 1966
Goldfish	static	96	25	7.5	18	20	44.49	Pickering & Henderson 1966
Guppy	static	24	25	7.5	18	20	49.86	Pickering & Henderson 1966
Guppy	static	48	25	7.5	18	20	49.86	Pickering & Henderson 1966
Guppy	static	96	25	7.5	18	20	39.19	Pickering & Henderson 1966
Rainbow	static	48	6.3	7.8	240	320	5.4	Brown et al. 1967
Rainbow	static	48	11.8	7.8	240	320	8.0	Brown et al. 1967
Rainbow	static	48	18.1	7.8	240	320	9.8	Brown et al. 1967
Rainbow	static	48	12.6	7.8	230	290	7.5	Mitrovic et al. 1968
Rainbow	static	48	16-22	9.4	-	-	4	Kariya et al. 1968
Carp	static	48	16-22	9.4	-	-	50	Kariya et al. 1968
Ayu	static	48	16-22	9.4	-	-	9	Kariya et al. 1968
Fathead	flow thru	48	15	7.8-8.0	218-230	-	41	Ruesink & Smith 1975
Fathead	flow thru	48	25	7.8-8.3	222-228	-	28	Ruesink & Smith 1975
Fathead	flow thru	96	15	7.8-8.0	218-230	-	36	Ruesink & Smith 1975
Fathead	flow thru	96	25	7.8-8.3	222-228	-	24	Ruesink & Smith 1975

A review of the important literature on selected heavy metals has been completed and summarized in our quarterly report for the final quarter of 1977. Based on the review, suggested values for "estimated safe concentrations" were presented in that report for nine metals, which also included tentative recommendations for ammonia, cyanide and hydrogen sulfide. Recommendations are reiterated in Section 4.5 of the present report.

Interpretation

Our search strategy has provided a high success rate in obtaining published information on environmental cycling, microbial degradation and toxicities of studied organic compounds. The searches have been less successful in identifying literature on procedures for extraction and analysis of individual organic compounds of interest in fish, primarily because very little has been published on these compounds.

Results from our comprehensive bibliographic reviews have emphasized one highly significant point: the absolute scarcity of published information concerning the environmental impacts related to the majority of organic compounds produced and potentially released during advanced in situ fossil fuel processes. Information is severely lacking on the toxicity, degradation and bioaccumulation of these compounds in the environment.

4.1.2 Work Proposed But Not Completed This Year

We have not completed the bibliographic search for all organic compounds which have been identified from advanced fossil fuel technology process water. Until we have results from further organic analyses of process water, approximately 50 organic compounds remain to be searched.

During the first quarter of next year we plan to complete the computerized bibliographic searches of: (1) the remaining identified organic compounds; (2) several other organic compounds (e.g., acetone) for which we hope to avoid much superfluous literature; (3) analytical methods (e.g., tissue extraction techniques); and (4) the literature published from 1965-1970 for all organic compounds so far searched. In addition, hand searches will be expanded covering literature cited sections of references we have acquired, while we continue to examine and summarize all literature acquired. Finally, we are preparing for publication in the first half of next year a bibliography on the published literature dealing with aquatic ecosystem cycling, uptake, toxicity, analytical methods, etc., of organic compounds known to occur in process waters from advanced fossil fuel technologies.

4.1.3 Recommendations For Work Next Year

We propose to continually update our bibliography by periodically conducting computerized data base searches for recently published literature. We additionally propose to expand our bibliographic search to review available literature on advanced control technologies appropriate for and possibly transferable to the treatment of process waters from advanced fossil fuel technologies.

4.2 Task 2: Toxicity Studies

4.2.1 Coal Gasification Studies (DeGraeve, R. Johnson, Bergman)

4.2.1.1 Work Completed This Year

During the period 15 April 1977 to 15 December 1977 we completed: (1) flow-through 96-hr acute bioassays exposing fathead minnows (Pimephales promelas) and rainbow trout (Salmo gairdneri) to phenol, catechol, resorcinol, o-cresol, m-cresol, p-cresol and naphthalene; (2) flow-through 48-hr acute tests with Daphnia pulex exposed to resorcinol, o-cresol, m-cresol and p-cresol; (3) static, acute bioassays exposing fathead minnows, rainbow trout and Daphnia to dilutions of condenser water obtained from LERC's Hanna 3 Environmental Burn; and (4) flow-through embryo-larval test with fathead minnow eggs and fry in Hanna 3 Environmental Burn condenser water. In addition we: (1) developed facilities and techniques for testing the predator-avoidance response of fish surviving sublethal concentrations at the termination of acute or long-term toxicity tests; (2) modified a proportional diluter to provide continuous flows to toxicant-avoidance chambers; and (3) constructed a prototype of the toxicant-avoidance chamber to be used in conjunction with the modified diluter.

Methods Synopsis

Flow-through acute bioassays were conducted according to the procedures appended to the 15 April 1977 to 5 June 1977 Quarterly Progress Report. Static, acute toxicity bioassays were performed in 3-l beakers; the toxicant and dilution water were combined prior to the introduction of the test animals. Typically, we conducted these studies as range-finding tests for future flow-through studies.

Embryo-larval exposure bioassays followed the procedures advanced by McKim (1977); fathead-minnow eggs and the hatched fry were exposed to condenser-water concentrations through 30 days of age. Egg survival and hatchability plus fry survival and growth were monitored through the study.

Predator-avoidance chambers were modified from those suggested by Goodyear (1972) to test the predator avoidance of fish previously exposed to sublethal levels of toxicant.

Toxicant-avoidance tanks are 30 cm X 60 cm aquaria modified to provide a clean-water contaminated-water choice to the test fish.

Results

Table 4.2-1 presents the results of all our flow-through acute bioassays; Table 4.2-2 provides survival data at individual concentrations for each bioassay. Results from static, acute bioassays with rainbow trout, fathead minnows and Daphnia exposed to condenser water (Table 4.2-3) were used to determine test concentrations for subsequent flow-through acute bioassays and embryo-larval exposure studies.

Although analysis of the embryo-larval condenser water bioassay is still in progress, the hatchability of fathead-minnow eggs has been analyzed (Table 4.2-4).

Table 4.2-1. Summary of acute, flow-through bioassay results for rainbow trout (Salmo gairdneri), fathead minnows (Pimephales promelas) and invertebrates (Daphnia pulex) exposed to six phenolic compounds.^a

Compound	Species ^b	Number of organisms per concentration	Highest test concentration (mg/l)	TL ₅₀ (mg/l)	Observations
Phenol	Rainbow trout	10	10	None	At 10, 5 and 2.5 mg/l: hyperactivity, gasping at the surface, rapid operculatation, and excitable.
	Fathead minnows	20	10	None	At 10 mg/l: fin-twitching, gasping at the surface, rapid operculatation, and excitable.
Phenol	Rainbow trout	10	100	8.9	At 12.5, 6.3 and 3.1 mg/l: hyperactivity, gasping at the surface, rapid operculatation, and excitable.
	Fathead minnows	20	100	67.5	At 50, 25 and 12.5 mg/l: fin-twitching, gasping at the surface, rapid operculatation, and excitable.
Catechol	Rainbow trout	10	100	8.9	At 6.25 mg/l: hyperventilation, rapid movements, extremely sensitive.
	Fathead minnows	20	100	3.5	At 3.12 mg/l: loss of equilibrium.
Resorcinol	Rainbow trout	20	100	None	20% mortality at 100 mg/l.
	Fathead minnows	20	100	>100	At 50 mg/l: rapid operculatation, sensitive to disturbance.
	Daphnia	10	100	None	Test duration was 24 hours. 90% survival at 100 mg/l, although at 100 and 50 mg/l Daphnia were immobilized.
o-Cresol	Rainbow trout	10	100	8.4	At 5.9 mg/l: hyperactivity.
	Fathead minnows	10	100	18.2	At 11.8 mg/l: some hyperactivity and twitching.
	Daphnia	10	100	None	Test duration was 48 hours. Insufficient mortality to calculate TL ₅₀ .

Table 4.2-1. (Continued)

Compound	Species ^b	Number of organisms per concentration	Highest test concentration (mg/l)	TL ₅₀ (mg/l)	Observations
m-Cresol	Rainbow trout	10	100	8.9	At 6.23 mg/l: hyperactivity, rapid operculation, sensitive to slight disturbances.
	Fathead minnows	10	100	55.9	At 24.9 mg/l: aimless movements, gasping at the surface, some loss of equilibrium.
	Daphnia	10	100	None	Test duration ^a was 24 hours. Insufficient mortality to calculate TL ₅₀ .
p-Cresol	Rainbow trout	10	100	8.6	At 6.25 mg/l: fish lethargic.
	Fathead minnows	10	100	28.6	At 25 mg/l: signs of stress continued for two days but then reduced.
Naphthalene	Daphnia	10	100	22.7	Test duration was 48 hours.
	Rainbow trout	10	5.6	2.25	Immediate loss of equilibrium at 5.6 and 3.1 mg/l. Lethargic behavior at 1.5 and 0.71 mg/l.
	Fathead minnows	20	5.6	4.9	Immediate loss of equilibrium at 5.6 mg/l. Lethargic behavior at 3.1, 1.5 and 0.71 mg/l.

^aAll acute tests were 96 hours unless specified, as for Daphnia pulex.

^bRainbow trout mean weight 6.0g and mean length 79mm; fathead minnows mean weight 1.6g and mean length 49mm.

Table 4.2-2. Summary of survival (percent) by concentration for flow-through acute toxicity tests.

Compound tested	Species	Concentration in mg/l (survival)							
		10	5	2.5	1.25	0.63	0.31	0.16	0.00
Phenol	Rainbow trout	100 (100)	50 (100)	25 (100)	12.5 (100)	6.25 (100)	3.12 (100)	1.56 (100)	0.00 (100)
Phenol	Fathead minnow	100 (100)	50 (100)	25 (100)	12.5 (100)	6.25 (90)	3.12 (100)	1.56 (100)	0.00 (100)
Phenol	Rainbow trout	100 (0)	50 (0)	25 (0)	12.5 (0)	6.25 (100)	3.12 (100)	1.56 (100)	0.00 (100)
Phenol	Fathead minnow	100 (10)	50 (80)	25 (90)	12.5 (90)	6.25 (100)	3.12 (100)	1.56 (100)	0.00 (100)
Catechol	Rainbow trout	100 (0)	50 (0)	25 (0)	12.5 (0)	6.25 (100)	3.12 (100)	1.56 (100)	0.00 (100)
Catechol	Fathead minnow	100 (0)	50 (0)	25 (0)	12.5 (0)	6.25 (0)	3.12 (60)	1.56 (100)	0.00 (100)
Resorcinol	<u>Daphnia pulex</u>	100 (100)	50 (100)	25 (90)	12.5 (90)	6.25 (90)	3.12 (80)	1.56 (90)	0.00 (90)
Resorcinol	Rainbow trout	100 (80)	50 (100)	25 (100)	12.5 (100)	6.25 (100)	3.12 (100)	1.56 (100)	0.00 (100)
Resorcinol	Fathead minnow	100 (50)	50 (95)	25 (100)	12.5 (100)	6.25 (100)	3.12 (100)	1.56 (100)	0.00 (100)
Para-cresol	Rainbow trout	91 (0)	46 (0)	23 (0)	11.3 (0)	5.6 (90)	2.8 (100)	1.4 (100)	0.00 (100)
Para-cresol	Fathead minnow	91 (0)	46 (10)	23 (70)	11.3 (100)	5.6 (100)	2.8 (100)	1.4 (100)	0.00 (100)
Ortho-cresol	Rainbow trout	94 (0)	47 (0)	24 (0)	11.8 (0)	5.9 (100)	2.9 (100)	1.5 (100)	0.00 (100)
Ortho-cresol	Fathead minnow	94 (0)	47 (20)	24 (20)	11.8 (100)	5.9 (100)	2.9 (100)	1.5 (100)	0.00 (100)

Table 4.2-2. (Continued)

Compound tested	Species	Concentration in mg/l (survival)							
Meta-cresol	Rainbow trout	98 (0)	49 (0)	25 (0)	12.5 (0)	6.2 (100)	3.1 (100)	1.6 (100)	0.00 (100)
Meta-cresol	Fathead minnow	98 (0)	49 (60)	25 (100)	12.5 (100)	6.2 (100)	3.1 (100)	1.6 (100)	0.00 (100)
Meta-cresol	<u>Daphnia pulex</u>	98 (70)	49 (90)	25 (100)	12.5 (100)	6.2 (90)	3.1 (80)	1.6 —	0.00 (90)
Naphthalene	Rainbow trout	5.6 (0)	3.1 (10)	1.5 (100)	0.71 (100)	0.44 (100)	0.15 (100)	0.10 (100)	0.00 (100)
Naphthalene	Fathead minnow	5.6 (35)	3.1 (100)	1.5 (100)	0.71 (100)	0.44 (100)	0.15 (100)	0.10 (100)	0.00 (100)

Table 4.2-3. Summary of acute, static bioassay results for rainbow trout (Salmo gairdneri), fathead minnows (Pimephales promelas) and the invertebrate Daphnia pulex.

Toxicant tested	Species	Test duration	Exposure concentrations	Number of organisms per concentration	TL ₅₀	Observations
Hanna 3 condenser water	Fathead minnows (adult)	96-hr.	5.0, 1.0, 0.1 and 0.05% and well water control	5	0.075% condenser water	5 and 1 percent exposed fish were dead in 10 minutes; 0.1% were all dead in 6 hrs.
Hanna 3 condenser water	Fathead minnows (fry)	48-hr.	5.0, 1.0 and 0.1% and well water control	8	<0.1% condenser water	5 and 1 percent exposed fish were dead in 15 minutes; 0.1% were all dead in <11 hrs.
Hanna 3 condenser water	Rainbow trout (juvenile)	48-hr.	1.0, 0.05 and 0.1% and well water control	5	<0.1% condenser water	1.0 and 0.5% exposed fish were dead in 10 minutes; 0.1% were dead in 11 hrs.
Hanna 3 condenser water	<u>Daphnia pulex</u>	96-hr.	10.0, 5.0, 1.0, 0.5 and 0.1% and well water control	10	0.183% condenser water	10 and 5% exposed <u>Daphnia</u> were dead immediately; 1% were dead in 1 hr.; 0.5% were dead in 12 hrs.

Table 4.2-4. Hatchability of fathead minnow (Pimephales promelas) eggs incubated in Hanna 3 condenser water diluted with well water.

Condenser water dilution (%)	Number of incubation trials ^A	Mean egg hatchability (%)
0.08	8	19 ^B
0.04	8	63 ^B
0.02	7	73
0.01	7	70
0.005	6	79
0.0025	5	76
0.0013	5	81
0 (control)	4	84

^AEach incubation trial consisted of exposing 50 eggs to the condenser water dilution shown.

^BHatchability significantly different ($p = 0.05$) from control (well water).

Statistical differences among eggs incubated in condenser water dilutions and the controls were determined by the standard t-test. This bioassay indicates that condenser water concentrations of 0.04% and higher have negative effects on the hatchability of fathead-minnow eggs.

Interpretation

The toxicity data accumulated this past year contributes to a characterization of in situ coal gasification condenser water and its constituents. Since the untreated condenser water has obvious toxic effects (egg hatchability reduced at concentrations > 0.04%), it is important that we identify the constituents causing those effects.

For those species tested the single compound acute mortality data indicates that all of the phenolic compounds we have assayed except for resorcinol, produced similar mortality responses. The wide disparity of TL₅₀ values between resorcinol and catechol, both isomers of dihydroxybenzene, can perhaps be explained by the fact that catechol (1,2-dihydroxybenzene) is easily oxidized to o-benzoquinone, which produces a dark-colored solution in water. We observed a similar dark-color formation during our catechol test, and 96-hr TL₅₀ values of 8.9 mg/l for rainbow trout and 3.5 mg/l for fathead minnows. Resorcinol (1,3-dihydroxybenzene) cannot be oxidized to a corresponding m-benzoquinone, however, and remained in the form in which it was dosed during the test. Since the resorcinol TL₅₀ values of > 100 mg/l for rainbow trout and 100 mg/l for fathead minnows were considerably higher than those observed for catechol, it suggests that benzoquinones are much more toxic to the test species than dihydroxyphenols. If our theory is true; when we test the toxicity of hydroquinone (1,4-dihydroxybenzene) during the next quarter we should see that compound oxidized to p-benzoquinone, producing a yellow solution and a much higher toxicity than that of resorcinol.

The observed toxicity of phenolics has particular significance to this project. Our liquid chromatography analyses of condenser water from the Hanna 3 Environmental Burn showed a compound tentatively identified as phenol at a concentration of 2,300 mg/l (see Appendix A). Since our flow-through bioassays produced TL₅₀ values for phenol with rainbow trout and fathead minnows of 8.9 and 67.5 mg/l, respectively, it appears that phenol contributes significantly to the toxicity of the untreated condenser water. An embryo-larval test with phenol (Section 4.2.1.2) should help to define the importance of phenol in the toxicity of condenser water.

Naphthalene, a compound also found in condenser water, is the most acutely toxic model compound tested to date with a TL₅₀ of 2.25 and 4.9 mg/l to rainbow trout and fathead minnows, respectively (Table 4.2-1).

Responses of Daphnia pulex, an invertebrate which is typically highly sensitive to toxicants, to the model compounds and the condenser water were unexpected (Tables 4.2-1 and 4.2-3). With the exception of the test with p-cresol, D. pulex was less sensitive in all bioassays than were rainbow trout and fathead minnows. Reasons for these responses remain unclear, but we will continue to investigate this phenomenon during future bioassays.

4.2.1.2 Work Proposed But Not Completed This Year

Work Not Completed

Of the work we proposed to complete for Task 2 toxicity studies during 1977, the following tasks were not completed:

- 1) Hanna 3 condenser water short-term flow-through bioassays.
- 2) Hanna 4 condenser water short-term flow-through bioassays.
- 3) Hanna 4 long-term test.
- 4) Preliminary and short-term bioassays on condenser water fractions.
- 5) Long-term studies on a model compound.

Schedule for Completion

In addition to work we have proposed for 1978 (see Sect. 4.2.1.3) we will complete our proposed 1977 toxicity studies according to the following schedule:

- 1) Hanna 3 short-term flow-through bioassays will be completed during December 1977.
- 2) Hanna 4 short-term flow-through bioassays will be conducted when sufficient condenser water is available, probably early in 1978.
- 3) The Hanna 4 long-term test for 1977 has been replaced by a Hanna 3 long-term test (see Section 4.2.1.1), which will be complete including data analysis and interpretation by January 30, 1978. Initiation of the Hanna 4 long-term test depends on the availability of condenser water from LERC; this test should begin in late January 1978.
- 4) Hanna 3 condenser water was not fractionated in 1977, thus these short-term tests will not be completed until we have enough condenser water to fractionate and test; these tests will be done now with Hanna 4 water probably in May and June.
- 5) A long-term test on a single compound (a phenolic) is scheduled to follow the Hanna 4 long-term test, probably in May or June 1978.

4.2.1.3 Recommendations For Work Next Year

Contractor

In 1978 our coal gasification toxicity studies will center around acute and embryo-larval bioassays on the condenser water and on model compounds. When Hanna 4 condenser water is available (probably January or February) we will conduct embryo-larval bioassays and then a flow-through acute test with this water. For the embryo-larval bioassay we will expose rainbow trout in procedures similar to those used in the fathead minnow long-term bioassay with Hanna 3 condenser water. Using a different species will provide a broader perspective of the long-term effects of the condenser water.

Hanna 4 condenser water fractions will be tested in static, acute toxicity bioassays with fathead-minnow larvae. Since larval exposure tests have proven to be highly sensitive, these results will provide us with information that will help identify those fractions most responsible for the observed toxicity with the condenser water.

The acute toxicity of six more model compounds, several of which will be multiple-ring, will be assayed using rainbow trout, fathead minnows and D. pulex. Based upon the results of these acute tests and those which we have already performed, we will select two model compounds (one for the 1977 test which was not completed and one for the proposed 1978 work plan) on which to conduct embryo-larval exposure bioassays. One will probably be phenol, due to its high concentrations in the condenser water. We will also select two previously tested model compounds and repeat the flow-through acute bioassays in softer water test conditions. These results will enable us to study how the toxicity of these organics is affected by water hardness.

Late in 1978 we plan to analyze the acute toxicity of ground water which has infiltrated the site of the Hanna 3 Environmental Burn. These tests will provide information about the potential long-term effects of in situ coal gasification processing.

The acute and long-term toxicity of oil shale retort water to rainbow trout and fathead minnows has been evaluated (Section 4.2.2.1). The data obtained from these tests will be further evaluated to determine if additional bioassays are necessary. If so, we will conduct those tests during the summer of 1978. In addition LERC has agreed to provide oil-shale retort water fractions for static, acute tests with fathead minnow fry. The results of these tests will help to determine the most toxic components of the retort water.

LERC has also agreed to provide process water from the Vernal, Utah Tar Sands Recovery operation. Depending on the volume supplied, we will conduct either flow-through acute tests with fathead minnows and rainbow trout or static, acute tests with fathead minnow fry.

Outside Support

For us to complete the toxicity objectives outlined for 1978, LERC should provide the following:

- 1) Hanna 4 condenser water (140 l minimum).
- 2) Hanna 3 ground water (required amount dependent upon the toxicity of this water).
- 3) Fractions of Omega-9 retort water (approximately one liter of each fraction).
- 4) Tar Sands Recovery process water from Vernal, Utah (amount required is dependent upon the toxicity of this water, probably a minimum of 5 gallons for flow-through acute tests).

4.2.2 Oil Shale Studies (Anderson)

4.2.2.1 Work Completed This Year

Flow-through and static 96-hr. toxicity bioassays on oil shale retort water (Omega-9) were performed using rainbow trout, Salmo gairdneri, and fathead minnows, Pimephales promelas. Results were analyzed using a computer program from U.S. EPA, Duluth, Minnesota. Avoidance studies using rainbow trout fry and several dilutions of Omega-9 water were performed.

Serum levels of SGOT, SGPT, CPK, LDH, LAP, ALP (Respectively: serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, creatine phosphokinase, lactic acid dehydrogenase, leucine aminopeptidase, alkaline phosphatase) and acid phosphatase were analyzed in rainbow trout exposed to several dilutions of Omega-9 water during flow-through experiments. Hemoglobin and packed cell volume were measured in blood from rainbow trout exposed to Omega-9 water in flow-through and static experiments. Blood was also sent to an outside lab for a comparative assay and results were obtained for CPK, calcium, LDH, inorganic phosphorus, creatinine, SGPT, SGOT, ALP, total bilirubin, sodium, potassium, cholesterol, chloride, total protein, globulin, urea nitrogen, and glucose.

Serum ALP was studied in rainbow trout to determine the relationship between ALP activity and duration of exposure to Omega-9 water, and between ALP activity and the concentration of Omega-9 water. ALP kinetic studies were done on several tissues from Omega-9 exposed and control rainbow trout. Aldehyde dehydrogenase (ALDH) activity in subcellular fractions of rainbow trout liver and several microsomal oxidative enzymes were studied in Omega-9 exposed and control fish.

Equipment and supplies for the histology laboratory were purchased and prepared for use. Histochemical and histopathological methods were tested and adapted for use on fish tissues. Initially, several chronically exposed rats were examined grossly and histologically. Various tissues from rainbow trout (Omega-9 exposed and controls) were examined for histopathologic lesions using a routine hematoxyline and eosin staining technique. The kidney and liver from Omega-9 exposed and control rainbow trout were histochemically stained for alkaline phosphatase activity.

Spectrophotometric differences among barrels of Omega-9 water were examined.

Methods Synopsis

Rainbow trout, Salmo gairdneri, and fathead minnows, Pimephales promelas, were used to determine 96-hr toxicity levels in static bioassays. Rainbow trout were also used for 96-hr flow-through bioassays.

In 96-hr static toxicity bioassays fish were placed in tanks containing 50L of dechlorinated tap water (temperature maintained by a water bath). After an adjustment period of at least 18 hrs., reported quantities of Omega-9 water were added to the tanks. The percentage survival at 96 hr was recorded. In the 96-hr flow-through toxicity tests the fish were exposed to unaerated dilutions of Omega-9 water, except for two tests when aeration was provided in the diluter box. Water chemistries were performed on specified tests. The diluter used in the flow-through tests was designed after Mount and Brungs (1967). The fish were not fed during the static and flow-through tests. The 96-hr TL₅₀ results were analyzed using a program obtained from U.S. EPA, Duluth.

Avoidance tests were conducted in a chamber constructed according to Goettl and Edde (1976). Twelve rainbow trout fry were given a 20 minute adjustment period to the avoidance chamber with fresh dechlorinated tap water in all compartments and then a 10 minute adjustment period with an Omega-9 dilution flowing on one side of the chamber. After the adjustment periods, counts were made every 30 seconds for 20 minutes and the number of fish on each side was recorded. The maze was then flushed and the test repeated reversing the control and Omega-9 sides. Fry were used only once. Flow rate during the test was 130ml/min.

Enzyme activities, packed cell volume, and hemoglobin were determined in the blood of rainbow trout from the 96-hr flow-through toxicity bioassays, using standard clinical chemistry techniques (CPK - Hess et al. 1968; ALP - Bessey et al. 1946; LAP - Martinak et al. 1964; SGOT - Karmen 1955; SGPT - Wroblewski et al. 1956; LDH - Cabaud et al. 1958). Blood for the assays was obtained by caudal amputation, collected in glass test tubes, allowed to clot, and centrifuged at room temperature. Several serum parameters were also tested using a Hycel Auto-Analyzer.

Whole liver homogenates from rainbow trout used in the 96-hr flow-through toxicity tests were assayed for SGOT, SGPT, CPK, LAP, ALP, and acid phosphatase activity using standard assay procedures. Proteins were determined by the biuret method.

Subcellular fractionation of rainbow trout liver homogenates (Statham et al. 1977) from fish used in 96-hr flow-through toxicity tests preceded analysis for ALDH activity, using the method of Lebsack et al. (1977). Alkaline phosphatase activity in several different tissues from rainbow trout used in 96-hr flow-through toxicity tests was determined by the method of Petersen (1974).

Histochemical study of ALP activity in tissues from rainbow trout exposed to Omega-9 water, was done by a modified Gomori calcium cobalt technique (Pearse 1968). Histopathologic examinations of tissues were performed using paraffin sections stained with a routine hematoxylin and eosin method (Luna 1968). Hepatic enzymes were assayed according to Pedersen et al. (1974).

Results

Tables 4.2.2-1, 2, and 3 summarize the test conditions and results from the 96-hr static and flow-through bioassays. Rainbow trout, Salmo gairdneri, were the most sensitive species in the static and flow-through bioassays on Omega-9 retort water. The 96-hr flow-through bioassay for 11-18cm rainbow trout gave a TL_{50} 95% confidence interval of 3.79-3.91 ml/L (0.379-0.391%) as determined by a binomial test (Figure 4.2.2-1). There is a marked decrease in the dissolved oxygen concentration in the water containing Omega-9 as compared to control water (Table 4.2.2-4). Others working with Omega-9 water have also reported that it decreases dissolved oxygen. In order to determine that the toxicity was not due to insufficient oxygen, experiments were performed with fewer fish and increased oxygenation in the tanks.

Table 4.2.2-5 shows the avoidance chamber results for 1977. As noted by Goettl and Edde (1976), the fish exhibited a marked side preference. Averaging the side preferences shows that the rainbow trout avoid the Omega-9 water at concentrations higher than the TL_{50} .

Tables 4.2.2-6, 7, 8, 9, 9a, and 9b summarize the blood and serum assays for fish exposed to Omega-9 water and control fish. Alkaline phosphatase activity showed the most consistent response to Omega-9 exposure, decreasing with both increased length of exposure and increased concentration of Omega-9.

The kinetic studies of tissue alkaline phosphatase activity in rainbow trout are reported in Table 4.2.2-10. The K_m and V_{max} for alkaline phosphatase do not seem to change.

Aldehyde dehydrogenase (ALDH) activity is being investigated in rainbow trout liver as a possible sensitive bioassay procedure. Deitrich et al. (1977) have found that tetrachlorodibenzo-p-dioxin induces rat liver aldehyde dehydrogenase. The same chemical induces aryl hydrocarbon hydroxylase, which is claimed to be a sensitive bioassay in fish (Payne, 1976). There appears to be at least one ALDH activity in the cytosol and one in the microsomes. The biphasic nature double-reciprocal graphs indicate the presence of two ALDH activities in the mitochondria. The apparent K_m 's for ALDH in the mitochondrial fraction are 0.6 mM and 0.04mM. The K_m for ALDH in the cytosol is 0.7mM and in the microsomal fraction it is 1mM. Acute exposure to Omega-9 water possibly inhibits ALDH activity.

There does not appear to be any difference in aryl-hydrocarbon hydroxylase activity when the rainbow trout were exposed to Omega-9 water.

Figures 4.2.2-2, 3, and 4 show the results of the spectrophotometric scans of several Omega-9 samples. These scans show a peak at 655nm in the blue-green Omega-9 which was not present in the normal Omega-9. The blue-green Omega-9 lacked the side hump exhibited at 204nm-216nm in the normal Omega-9. The ultraviolet scans of samples from 4 barrels of Omega-9 show variations in the intensity of absorption at almost all wavelengths.

The hematoxylin and eosin procedure was not sensitive enough to detect morphologic changes in the acutely exposed rainbow trout or the chronically exposed Holtzmann rats examined. Histochemical examinations of alkaline phosphatase activity in Omega-9 exposed and control rainbow trout seem to show different staining intensities.

Table 4.2.2-1 Percent Survival of Rainbow Trout (Salmo Gairdneri) exposed to Omega-9 Retort Water in 96-Hour Static Tests.

Test No.	Initial Temperature (°C)	TL50 % Retort Water	Exposure Concentration (% initial retort water)									
			0	.06	.07	.08	.09	.10	.11	.12	.13	.14
3*	13	+ >.08	100	100	100	100						
4*	8	+ >.09	100	100	100	100	90					
5*	7	+ >.08	100		100	100						
6*	7	+ >.15	100					100		100		100
7*	7	+ >.18	100									
8*	7	+ <.22	100									
9*	7	+ <.21	100									
10*	8	+ >.23	100									
11*	9	+ <.22	100									
12*	8	+ >.14	100			90		100		90		80
13*	8	+ >.18	100									
14*	9	+ <.18	100									
16*	11	+ >.18	100						90	100		
17*	10	+ <.18	100									
19*	11	+ >.20	100									
1A#	10	+ >.19	100						100		100	
1B#	10	+ >.27	100									

+ cannot be calculated

3-7 cm fish, 10 fish per tank

* 8-20 cm fish, 25 fish per tank

(Continued Next Page)

Table 4.2.2-1 Continued

Test No.	.15	.16	.17	.18	.19	.20	.21	.22	.23	.24	.25	.27
6	80											
7		90		70	50	70						
8					40	0	60	20				
9				100	30	10	70					
10						60	50	60	60			
11								40	0	0	0	
13	90	100	80	80								
14				20		10	0	0				
17			100	20								
19						30	50					
1A	100		92		96							
1B					84		88		76		80	92

Table 4.2.4-2 Percent Survival of Fathead Minnows (Pimephales promelas) exposed to Omega-9 Water for 96-Hour Static Tests.

Test No.	No. Fish Exposed to each Concentration	Initial Temperature (°C)	TL50 % Retort Water	Exposure Concentration (% Retort Water)							
				0	.17	.19	.21	.23	.25	.27	.29
2A	50	12.5	+ >.290	98	100	100	100	98	100	92	96
2B	40	14.0	~ .350	100							
2C	25	15.5	+ <.330	100							
2D	20	14.0	~ .301	100							
2E	25	15.0	+ <.290	100							

+ cannot be calculated

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Table 4.2.4-2 Continued

Test No.	.30	.31	.32	.33	.34	.35	.36	.40
2A								
2B			80				45	5
2C				36	4	8	16	
2D	40	60	25	5				
2E	8	4	4					

Table 4.2.2-3 Percent Survival of Rainbow Trout (Salmo Gairdneri) exposed to Omega-9 Water in 96-Hour Flowthrough Tests.

Test No.	Test Conditions	Fish Length (cm)	TL ₅₀ % Retort Water	Percent Survival							
				Exposure Concentration (% Retort Water)							
				0	.078	.104	.156	.208	.313	.329	.357
4	#	7-20	+ < .625	100	100		100		82		
5	#	7-20	~ .294	100		100		100			
8	#	13-25	+ > .313	100	96		100		88		
9	#	10-25	+ > .329	100						88	
10	#	7-25	+ > .357	100							84
11	#	7-20	+ > .379	96							70
12	#	13-18	+ < .417	96							
14	@\$	13-18	+ < .391	100							
1A	#	2-3		100			100		100		
1B	#	2-8	+ > .391	100							
1C	*#	2-8	+ > .417	100							
1D	#	2-8	+ < .625	100							
1E	#	3-12	+ > .481	100							
1F	\$	3-12	+ < .543	100							
1G	*\$	6-10	+ < .510	100							
1J	@%	15-20	+ < .500	100							

+ - cannot be calculated by available computer program

@ - aeration provided in dilutor

* - water chemistries performed

- 50 fish in each concentration

\$ - 25 fish in each concentration

% - 10 fish in each concentration

(Continued Next Page)

Table 4.2.2-3 Continued

Test No.	.379	.391	.417	.481	.500	.510	.543	.625
4			0					0
5								
8								
9								
10								
11	70							
12			30					
14		0						
1A								0
1B		100						
1C			100					
1D								2
1E				96				
1F							12	
1G						24		
1J					0			

Figure 4.2.2-1
TL₅₀ for 11-18cm Rainbow Trout

Table 4.2.2-4 Typical Water Chemistry Results from Flowthrough Bioassay

Chemistry	Dechlorinated Tap Water	Omega-9 0.417%	Omega-9 0.510%
Hardness - ppm	210	190	
Dissolved Solids - ppm	96	240	
Sulfates - ppm	3	27	
Nitrates - ppm	4.1	5.2	
pH	7.3		7.7
Alkalinity - ppm	134		197
Conductivity	320		520
CO ₂ - ppm	15.7		9.83
Dissolved Oxygen ppm (% saturation)			
Day 1	4.0 (51)		4.6 (59)
Day 2	4.3 (55)		3.7 (47)
Day 4	4.7 (62)		4.2 (55)
Temperature °C			
Day 1	15		15
Day 2	15		15
Day 4	16		16

Table 4.2.2-5 Avoidance Chamber Results*

% Omega-9	Number of Fish				Average Control vs Omega-9	
	Control vs Omega-9 (right)	Control vs Omega-9 (left)	Control vs Omega-9 (left)	Control vs Omega-9 (right)		
0.4	4	7	7	5	6	6
0.5	5	7	4	8	5	7
0.6	7	5	4	8	5	7
1.0	5	7	9	3	7	5
1.0	7	5	6	6	7	5
2.0	10	2	6	6	8	4
2.5	7	5	8	4	8	4
4.0	11	1	7	5	9	3
4.3	10	2	7	5	8	4
5.0	10	2	7	5	8	4
5.6	8	4	8	4	8	4
6.7	8	4	6	6	7	5
6.7	6	6	9	3	8	4

* Water temperature 8°C - 9°C

Table 4.2.2-6 Hemoglobin and packed Cell Volume in Rainbow Trout Exposed to Omega-9 Water

Exposure Concentration % Omega-9	Hemoglobin mg %	Packed Cell Volume %
0.0	7.2 $\pm$ 2.5	37 $\pm$ 14
Static Tests		
0.10	6.1 $\pm$ 1.1	33 $\pm$ 6
0.12	6.7 $\pm$ 1.6	32 $\pm$ 11
0.14	7.2 $\pm$ 1.6	33 $\pm$ 7
0.16	7.2 $\pm$ 1.8	34 $\pm$ 6
0.17	5.9 $\pm$ 2.1	29 $\pm$ 9
0.18	7.4 $\pm$ 1.4	36 $\pm$ 6
0.19	5.9 $\pm$ 2.0	36 $\pm$ 7
0.20	6.1 $\pm$ 2.1	36 $\pm$ 10
Flow-through Tests		
0.357	8.4 $\pm$ 2.4	45 $\pm$ 13
0.329	8.4 $\pm$ 1.2	47 $\pm$ 8
0.313	7.1 $\pm$ 1.9	36 $\pm$ 11
0.208	4.6 $\pm$ 1.5	31 $\pm$ 9
0.156	7.5 $\pm$ 2.2	48 $\pm$ 9
0.104	4.6 $\pm$ 1.6	35 $\pm$ 7

Table 4.2.2-7 Serum Enzymes in Rainbow Trout Exposed to Omega-9 Water in Flow Through Experimenting

% Omega-9	SGOT I.U.	SGPT I.U.	LDH I.U.	CPK I.U.	Acid P'tase I.U.	LAP I.U.
0.0	1747 ± 717	122 ± 82	11.658 ± 4280	1864 ± 1552	0.87 ± 0.48	6.4 ± 2.4
0.313	1133 ± 300	60 ± 28	7333 ± 2793	1542 ± 650	0.37 ± 0.15	5.1 ± 2.7
0.156	1000 ± 216	41 ± 23	6117 ± 630	1675 ± 565	0.41 ± 0.19	5.8 ± 2.5
0.078	1098 ± 188	46 ± 9	6533 ± 1425	2170 ± 709	0.46 ± 0.11	6.7 ± 2.0

Table 4.2.2-8 Serum Assays in Rainbow Trout Exposed to Omega-9 Water in Flow Through Experiments.

Assay	Control	Exposed for 96 Hour to 0.357% OMEGA-9 * Water
Creatinine mg/dl	3.6	1.93
Calcium mg/dl	11.9	9.97
LDH U/L	825	893
Inorganic Phosphorus mg/dl	15.5	14.5
CPK U/L	266	140.7
SGPT U/L	60	53.7
SGOT U/L	920	785
Alk. Phosphatase U/L	72.5	39.5
Total Bilirubin mg/dl	.8	.4
Sodium mEq/L	143.5	145.3
Potassium mEq/L	3.6	3.63
Cholesterol mg/dl	278	210.3
Chloride mg/dl	125	130.3
Total Protein gm/dl	3.1	2.23
Globulin gm/dl	1.7	1.27
Urea Nitrogen mg/dl	4	2.4
Glucose mg/dl	104	87

*Average of three experiments

Table 4.2.2.-9 Decrease in serum alkaline phosphatase activity with time[#].

Length of Exposure (hr)	<u>Alkaline Phosphatase Activity</u> [*]	
	Exposed ^{**} (IU)	Unexposed (IU)
24	30.90	36.24
48	18.37	36.74
72	7.01	35.07
96	3.34	35.48

* See Methods synopsis

** Exposed to retort water in a flow-through system at 0.379% Omega 9

Single determinations

Table 4.2.2-9a Decrease in serum alkaline phosphatase activity with increasing concentration of retort water (Experiment 1).

% Omega-9	Alkaline Phosphatase Activity* (IU)
0.0	79.5 [#]
.357	39.5 $\pm$ 9.01 ^{##}

* By Hycel Auto-Analyzer (Houston)

[#] Single Determination

^{##} Mean of 3 determinations $\pm$ S.D., 96 hr exposure

Table 4.2.2-9b Decrease in serum alkaline phosphatase activity with increasing concentration of retort water (Experiment II).

% Omega-9	Alkaline Phosphatase Activity [*] (IU)
0	70.14 $\pm$ 20.04 [#]
.078	53.44 $\pm$ 13.36 ^{##}
.156	48.43 $\pm$ 21.71 ^{##}
.313	35.07 $\pm$ 11.69 ^{##}

* See Methods synopsis

[#] Mean of 9 determinations $\pm$ S.D. (3 replicates x 3 samples each)

^{##} Mean of 6 determinations $\pm$ S.D. (2 replicates, 3 samples each)
96 hour exposure

Table 4.2.2-10 Kinetics of tissue alkaline phosphatase exposed to Omega-9 water.##

Tissue	% Omega-9	Km#	V _{max} **
Heart	0.288	0.29	0.10
	0.15	0.22	0.15
	0	0.41	0.22
Liver	0.28	1.19	0.15
	0.15	0.34	0.09
	0	0.24	0.06
Gills	0.28	0.23	0.09
	0.15	0.21	0.10
	0	0.26	0.08
Bone	0.28	0.29	0.33
	0.15	0.95	0.12
	0	0.25	0.03
Intestine	0.28	0.73	1.74
	0.15	2.18	1.15
	0	0.96	1.04
Stomach	0.28	0.34	0.12
	0.15	0.33	0.20
	0	0.37	0.11
Brain	0.28	0.47	0.07
	0.15	0.52	0.07
	0	0.57	0.10
Spleen	0.28	0.96	0.15
	0.15	0.36	0.16
	0	0.77	0.28

10^{-3} M para-nitro phenyl phosphate

** Change in OD per min.

All reported values are the result of two experiments.

Figure 4.2.2-2
Visible Spectrum Scans of Blue Green Omega-9 Water
and Normal Omega-9 (1:100 dilution)

Figure 4.2.2-3
Ultraviolet Spectrum Scans of Blue Green Omega-9 Water
and Normal Omega-9 (1:100 dilution)

Figure 4.2.2-4
Comparison of Four Barrels of Omega-9 Water

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Interpretation

Acute 96-hr toxicity bioassays show the potential effects of a worst case spill. The concentrations in these tests will not normally be found in the environment.

Additional control experiments are needed for further interpretation of the avoidance chamber results.

Levels of blood components and liver and serum enzyme activities show a large population variation, making it difficult to discern changes produced by Omega-9 water. However, in these tests serum ALP activity shows a marked difference in treated fish and may be a useful and sensitive assay of toxicity. Since the K_m and V_{max} show no apparent change, any changes in ALP activity are quantitative rather than qualitative. Preliminary experiments suggest that Omega-9 retort water does not induce aryl-hydrocarbon hydroxylase activity in rainbow trout. However, these experiments will be repeated because of the wide variation in control values (the variation is probably related to the wide population variation noted above).

While hematoxylin and eosin staining did not reveal histopathologic lesions, more sensitive procedures may detect morphologic changes in acutely exposed rainbow trout. Further study of tissues stained for ALP activity will be needed before conclusions can be drawn about the effects of Omega-9 exposure.

The source of the blue-green color in the Omega-9 water (the probable cause of the 655 nm peak) and the compounds involved in the change in intensity of absorption among barrels of Omega-9 will have to be determined by chemical methods not possible in available facilities.

4.2.2.2 Work Proposed But Not Completed This Year

A limited chronic flow-through study of the effect of several concentrations of Omega-9 water on the hatching and development of rainbow trout was started on 10 November 1977, using eyed eggs, 20 days post-fertilization. There is premature hatching and rupture without emergence with complete kill at 12 days at 5.35 ml/L. Weaker dilutions have shown no apparent differences from the control. Complete results of this test will be presented in the first quarterly report of 1978.

Acute bioassays proposed for Omega-9 fractions were not completed because fractionation work was not completed by LERC. These tests will be conducted during 1978 when the fractions are available.

4.2.1.3 Recommendations for Work Next Year

The biochemical, histochemical and pathological changes we are finding with the Omega-9 water will be investigated utilizing selected fish that have been exposed to organic compounds, fractions and other fossil fuel effluents. The specific activity of alkaline phosphatase and aldehyde dehydrogenase in various tissues will be determined.

Results of the flow-through study on the hatching and development of rainbow trout will be evaluated. The effects of several fractions and components of the oil shale effluent on light emitting bacteria will be studied. If the results are as promising as the preliminary reports indicate, this may be an inexpensive bioassay system that will not require copious volumes of toxicant. Electron microscopic studies of treated fish will be initiated in 1978.

According to Ungar et al. (1974), when aldehyde derivatives of indoleamines are incorporated into proteolipids of endothelial membranes, there is subsequent polymerization. This may play a triggering role in the fibrous proliferations observed in carcinoid tumors. The indole moiety may provoke the fibrous tissue overgrowth (Sandler 1968). We are currently studying the binding of aldehydes that are biologically important to fish liver tissue.

The concentration of 22 metals will be determined by plasma spectrophotometry in several tissues of rainbow trout exposed to Omega-9 water.

4.3 Task 3: Degradation Studies (Fannin, Meyer, Marcus, Parker, Bergman)

4.3.1 Work Completed This Year

Methods Synopsis

Both microcosm and bacterial systems were tested in degradation studies this year. The microcosm systems used, modified after Metcalf et al. (1971), proved unsuccessful for our degradation studies and were abandoned. Methods employed with this system are outlined in Section 4.4.

Details on the three different bacterial systems used to evaluate biodegradation of phenol and o-, m- and p-cresol have been presented in our quarterly report for 5 September to 5 December 1977. Briefly, System 1 consisted of 250-ml Erlenmeyer flasks, each containing basal medium, diatomaceous earth and the test compound. Each flask was fitted with condenser followed by volatile organic and CO₂ traps. This system was actively aerated during the experimental incubation period. System 2 consisted of flasks with contents as in System 1; however, the flasks were topped only with cotton plugs and incubated on a rotary shaker without aeration. System 3 was identical to System 2 except that the flasks were not shaken. With all three systems, the flasks (including the condensers for System 1) with their contents were sterilized before use. Then the test compound was added to the flasks and bacteria were added to the test flasks; control flasks were kept sterile. The bacterial inoculum was 5ml of water from either an oil refinery settling pond (Sinclair, Wyoming) or a eutrophic lake (LaBonte Lake, Laramie, Wyoming). The systems were incubated in continuous light at constant temperatures during each experiment.

With all systems, samples were withdrawn at timed intervals with sterile syringes from both test and control flasks, preserved with CuSO₄ and pH adjustment, and analyzed by liquid or gas chromatography.

Results

Semilog plots of test compound concentration (log) versus time (linear) displayed three distinctive periods in each bacterial degradation experiment: (1) a lag period during which no appreciable degradation occurs; (2) an activation period when a small but increasing amount of breakdown occurs; and (3) a maximum degradation period. As explained in our final quarterly report for 1977, the degradability of a compound can be most reproducibly determined by calculations based on the maximum degradation period. From the slope ($M_{\max}$) of the semilog plot for this period, the half-life during maximum degradation ($t_{dl/2\max}$) can be calculated by

$$t_{dl/2\max} = \frac{-\log 2}{M_{\max}}$$

Since $M_{\max}$ is a negative slope, the $t_{dl/2\max}$ value is positive.

Results of degradation experiments with phenol, and o-, m- and p-cresol and the three bacterial systems are presented in Table 4.3-1.

Table 4.3-1. Results of bacterial degradation tests of phenol and o-, m- and p-cresol.

Compound Test system-- bacterial source ^a	Lag period (Clock hrs)	Activation period				Maximum degradation period				
		Time (Clock hrs)	Δt_{act} (hrs)	M_{act}^b	r_{act}^c	Time (Clock hrs)	Δt_{max} (hrs)	M_{max}^b	r_{act}^c	$td_{\frac{1}{2}max}^d$ (hrs)
<u>Phenol</u>										
System 1-Sinclair	0-42	42-63	21	-0.011	0.75	63-69	6	-0.222	0.97	1.36
System 2-LaBonte	0-25	25-28.5	3.5	-0.065	0.97	28.5-30	1.5	-0.201	0.90	1.50
System 2-Sinclair	0-18	18-29	11	-0.020	0.94	29-44	15	-0.084	0.99	3.59
System 3-Sinclair	0-27	27-38	11	-0.003	1.0 ^e	38-76	38	-0.026	0.99	11.39
<u>o-cresol</u>										
System 1-Sinclair	0-20	20-25	5	-0.012	1.0 ^e	25-33.6	8.6	-0.251	0.92	1.20
<u>m-cresol</u>										
System 1-Sinclair	0-25	25-33	8	-0.039	0.99	33-38	5	-0.360	1.0 ^e	0.84
<u>p-cresol</u>										
System 1-Sinclair	0-20	20-28	8	-0.658	0.98	28-31	3	-0.566	0.91	0.53

^aTest Systems: 1 = aerated; 2 = shaken; 3 = static. Bacterial Sources: Sinclair = Oil refinery settling pond; LaBonte = local eutrophic lake.

^b M = slope = log concentration vs. time.

^c r = correlation coefficient.

^d $td_{\frac{1}{2}max}$ = maximum degradation half-time.

^e r calculated only on 2 points.

Interpretation

Although a maximum degradation rate was obtained from the results for phenol and the cresols, bacterial growth effects eclipse enzyme kinetic effects. Normalizing $t_{d1/2max}$ values to bacterial biomass will enable comparison of these kinds of results among compounds, and will also permit extension of the results to recommendations on design and sizing of treatment systems.

Although we cannot yet fully interpret these degradation test results until our normalization procedures are worked out, it is clear that phenol and the cresols are easily degraded by bacteria; this conclusion is supported by the literature. These compounds would be handled easily by activated biological sludge or treatment pond control systems, and would not tend to pose an environmental problem due to persistence.

4.3.2 Work Proposed But Not Completed This Year

Work Not Completed

Preliminary results have been obtained with four test compounds found in in situ fossil fuel technology process water. However, due to the need to normalize degradation rates to bacterial biomass, results from these experiments need to be refined in further experiments to produce degradation rate values which will permit comparison of rates among compounds.

Schedule For Completion

Methods for normalizing $t_{d1/2max}$ values to bacterial biomass (e.g., cell counts, absorbance, ATP assays) are under investigation. The results of these studies will be reported in the first quarter of 1978.

4.3.3 Recommendation For Work Next Year

During 1978, degradation tests are planned for eleven model compounds. These experiments will be designed to determine the biodegradability of each compound with the aim of providing necessary information transferable to future treatment designs for process waters from in situ fossil fuel technologies. All compounds will be aerobically tested, while some will be tested anaerobically.

The design of the bacterial systems used to determine biodegradability of model compounds will be standardized through a series of experiments currently underway and continuing into the first quarter of 1978. Experimental variables tested will include concentration of test compound, amount of bacterial inoculum, importance of solid substrate, level of light and temperature, etc. The standard test will include provisions to estimate bacterial biomass in order to normalize results.

4.4 Task 4: Bioaccumulation Studies (Meyer, Parker, Bergman)

4.4.1 Work Completed This Year

Work on determining the bioaccumulation potential of organics from in situ fossil fuel process waters in aquatic organisms centered mainly on the development and evaluation of methods. **Two main approaches were attempted for determining** bioaccumulation of individual organics: (1) chemical analysis of water and tissue extracts, and (2) uptake of radiolabeled organics in aquatic biota in laboratory microcosms.

For the chemical analysis procedures we achieved only moderate success, finding that routine pesticide extraction and clean-up procedures with gas chromatographic analyses were not completely satisfactory for polar aromatics such as phenol and cresol which were tested this year. **We therefore directed our efforts toward a** high performance liquid chromatograph (HPLC) technique which we believe will be more compatible with relatively polar extraction conditions. **Progress with this procedure** was delayed until acquisition and set-up of a Waters Associates Model 204 HPLC was completed in November.

The laboratory microcosm procedures were based on the methods of Metcalf et al. (1971), and these tests were meant to gain both bioaccumulation **and degradation** rate information. **But because of ambiguous results with the Metcalf microcosm,** especially for degradation rate determinations, these tests **were** abandoned in favor of a simpler degradation screening procedure explained in Section 4.3. **Based on** this decision the Metcalf microcosm approach for bioaccumulation determinations has also been set aside, at least temporarily, in favor of chemical extraction and HPLC analysis of exposed fish, from which concentration factors can be calculated. However, bioaccumulation data for ^{14}C -phenol **was obtained from microcosm experiments** as described below.

Methods Synopsis

A routine pesticide extraction and clean-up procedure was applied to rainbow trout and fathead minnows exposed to phenol in a 96-hr acute toxicity bioassay. Two to three grams of fish were homogenized with an appropriate organic solvent, the extract liquid was separated from the homogenized tissue through pyrex wool, and samples were injected into a Varian Model 600-D gas chromatograph (GC) equipped with a Carbowax 20M column and a flame ionization detector to quantify the amount of phenol accumulated in the fish.

Phenomena related to this basic procedure required investigation:

- 1) Isopropanol (IPA), benzene, chloroform (CHCl_3) and combinations of these solvents were evaluated as tissue extraction media.
- 2) Heating the tissue in the extraction solvent for 30 minutes at 55C following homogenization was compared to room temperature extraction.
- 3) Several solvents, hexane, benzene, toluene, IPA, acetonitrile (CH_3CN), methylene chloride (CH_2Cl_2) and tetrahydrofuran (THF), were compared for ability to elute phenol through 7mm ID X 5cm long Florisil clean-up columns.

- 4) Co-distillation of phenol in hexane, benzene, toluene, IPA, CH_3CN , CHCl_3 and CH_2Cl_2 during evaporative concentration procedures was investigated.

After adopting the HPLC procedure we began by evaluating various HPLC operating conditions for separation, detection and quantification of polar and non-polar organic standards. These methods are described in part in Appendix A as applied to water analyses. The methods will be developed further for extraction and analysis of organic tissue residues as described later in this section.

The Metcalf microcosm tests (Metcalf et al. 1971) were detailed in our Quarterly Report dated 5 September 1977. In brief, each microcosm consisted of a glass aquarium containing washed sand, standard water, 5 ml of bacterial seed water from a local eutrophic lake, mosquito fish, mosquito larvae, snails, water fleas, and algae. Carbon-14-labeled phenol was added to the aquarium, and at intervals up to 30 days samples of the microcosm components were analyzed using liquid scintillation counting.

Results

Phenol was extracted from rainbow trout exposed to 6.25 mg/l and fathead minnows exposed to 25 mg/l in a 96-hr flow-through acute toxicity test. Extracts injected into the GC without prior Florisil clean-up yielded quantifiable phenol peaks, but tissue components eluting after phenol prolonged the chromatographing time of each sample. Bioaccumulation factors of 1.0 - 1.5 were calculated for both species, but adequate extraction recovery data has not yet been obtained to correct these values for losses of phenol during the extraction.

No difference in solvent extracting strength was detected between 1:1 IPA: CHCl_3 and 1:1 IPA:benzene mixtures at room temperature, though heated extracts produced lowered phenol recovery with IPA: CHCl_3 and increased recovery with IPA:benzene. Co-distillation of phenol during tissue heating at 55C may have contributed to the unexpected recovery decrease of the IPA: CHCl_3 extract.

Phenol elution rates from Florisil columns increased with increasing solvent polarity. Hexane removed none, while 2.5 - 5.0 column volumes (5-10 ml) of benzene and toluene and less than 2.5 volumes of IPA, CH_3CN , CHCl_3 , CH_2Cl_2 and THF sufficiently eluted all the applied phenol. During simultaneously conducted evaporative concentration experiments co-distillation of phenol at 50C under gentle air flow was observed for all the solvents except THF, which was not tested.

Results from the aquatic microcosm experiment are given in Table 4.4-1 and 4.4-2. Because TLC separation of phenol degradation products was unsuccessful due to loss of labeled compounds from the plate by co-distillation with the solvent, we were unable to evaluate concentration factors based on phenol alone. Thus, the values reported in Tables 4.4-1 and 4.4-2 are expressed as phenol equivalents.

Interpretation

Routine pesticide extraction and clean-up procedures are not usable for phenolic compounds without modification. The extraction liquid containing the phenolics must be cleaned up before injection onto a GC column to prevent extended elution of and possible interference by residual tissue components, but traditional Florisil columns cannot adequately perform this. A relatively polar solvent (IPA or CH_3CN) elutes

Table 4.4-1. Mean concentration of phenol equivalents (phenol plus metabolites) in microcosm components.

Component	Day						
	0(-)	0(+)	6	12	19	25	30
Water (mg PE/l) ^a	0 ^b	0.26 ^c	0.062	0.054	0.024	0.026	0.026
Sand (μg PE/g)	0 ^b						
Mosquitoes (μg PE/g)	0 ^b		43	2.8			20
Daphnia (μg PE/g)						9.3	
Physa (μg PE/g)							45
Oedogonium (μg PE/g)							11
Gambusia (μg PE/g)							34

^aPE = Phenol equivalents (phenol and metabolites)

^bNot significantly above background activity.

^cCalculated from uniform mixing of ¹⁴C-phenol in microcosm water.

Table 4.4-2. Mean concentration factors (C_f) for phenol equivalents (phenol plus metabolites) in biotic microcosm components.

Component	C_f-1^a	C_f-2^b
Mosquitoes	76	267
Daphnia	36	109
Physa	173	600
Oedogonium	42	147
Gambusia	131	453

^aCalculated from initial phenol concentration of 0.26 mg/l.

^bCalculated from mean 30 day concentration of phenol (0.075 mg/l) except for *Daphnia* which was calculated from the mean 25 day concentration (0.085 mg/l).

the phenolics from Florisil in a reasonable number of column volumes, but some of the undesired tissue components will also elute at those solvent polarities and interfere with subsequent chromatography. In addition, traditional evaporative concentration of extract solutions reduces recovery by co-distilling the phenolic compounds with the organic solvent.

Hence, an alternative tissue extraction, clean-up and sample concentration system must be utilized. High pressure liquid chromatography has the potential to overcome these problems with versatile gel permeation and analytical chromatography systems that have been demonstrated in similar applications (see Sect. 4.4.3).

Though concentration factors on the basis of phenol alone could not be calculated from the microcosm experiment, it is likely that very little of the ^{14}C -label measured on day 30 represents phenol. Thus, concentration factors for phenol in this experiment are very low, possibly approaching 1.

4.4.2 Work Proposed But Not Completed This Year

Work Not Completed

Surviving fathead minnows and rainbow trout were frozen at the completion of all 96-hr acute toxicity bioassays performed this year and were stored for bioaccumulation analyses. Completion of these analyses and calculation of bioaccumulation factors awaits further evaluation of our extraction and HPLC clean-up and analysis procedure as described below.

Schedule For Completion

We are presently familiarizing ourselves with the operation of the recently acquired HPLC and are applying it to the analysis of the organic compounds in acute and chronic toxicity tests. Appropriate solvent flow rates and compositions are being determined that will be applicable to the later analyses of extracted residues from the fish exposed in these tests.

In the first quarter of 1978 we will continue to develop extraction and clean-up procedures for the volatile and polar compounds we tested in 1977. Bioaccumulation factors will be calculated for these tests by the end of the second quarter, 1978.

4.4.3 Recommendations For Work Next Year

Contractor

We plan to pursue a modification of the Pesticide Residue Clean-up Procedure described by Waters Associates (1975). Our proposed method will include extraction of whole fish by homogenization in Na_2SO_4 and CH_2Cl_2 , removal of particulate matter for injection onto HPLC columns, separation of the compounds of interest from the high molecular weight tissue components in the extract by HPLC gel permeation chromatography, and resolution and quantitation of these compounds by HPLC reverse-phase analytical chromatography. Selected radiolabeled compounds will be used to determine the recovery efficiency of the individual steps within this procedure and appropriate modifications will be employed as necessary.

After a suitable clean-up procedure has been developed, we plan to extract all the rainbow trout and fathead minnows saved from acute and chronic toxicity tests performed this past year. Fish surviving any tests in the coming year will be extracted at the completion of each test and analyzed for bioaccumulation of toxicants.

We also plan to investigate the applicability of certain physical chemical parameters of organic compounds as indicators of bioaccumulation potential in the manner of Carlson (Carlson and Caple 1977). Hansch-type structure-activity correlations will be calculated from published n-octanol/water partition coefficients and Hammett electronic substituent constants of those compounds identified in the process waters. HPLC relative retention times in reverse-phase acetone/water solvent programs will be used to approximate the n-octanol/water partitions of those compounds for which no appropriate published information exists. These theoretical predictions will be compared to the bioaccumulation factors determined in our extraction studies to evaluate the method's usefulness as a rapid screening technique to predict bioaccumulation potentials for the wide variety of organic compounds in the process waters.

Outside Support

We believe a practical HPLC extraction clean-up method can be adapted to our particular problem and the work already published is sufficient. However, the relationship of partition coefficients to biological responses has not been completely explored and will only be applied to specific compounds in our investigation.

We recommend that the important parameters, n-octanol/water partition coefficients and Hammett electronic substituent constants, available for those compounds identified in all fossil fuel process waters, be collected and published. Hansch-type correlations should be calculated and related to toxicity and bioaccumulation for an even wider range of compounds than we will test so the reliability of this technique as a mass-screening tool can be extended. In particular, reverse-phase HPLC gradient elution should be further investigated and refined as a rapid procedure for determining partitioning characteristics of compounds.

The EPA Environmental Research Laboratory-Duluth should consider funding separate projects by other investigators to obtain this kind of information. Also, as information becomes available we would benefit from consultation with staff members at EPA-Duluth for the purposes of applying these data to bioaccumulation prediction.

4.5 Task 5: Recommendations (Marcus, Bergman)

Tentative values for estimated safe concentrations in aquatic ecosystems for 13 selected chemicals from in situ process waters are presented in Table 4.5-1. These values are tentative estimates for maximum concentrations permissible in receiving waters for compounds considered individually. Synergistic effects have not been considered as yet in these recommendations. Selection of the estimated safe levels was based on our survey of the literature for each chemical. Specific criteria on which these values were selected have been presented in our quarterly reports for the third and fourth quarters of 1977. These chemicals were chosen for emphasis at this time because of their likely presence in process water and the availability of published information.

Tentative values for estimated safe concentrations for the seven single compounds tested in our Task 2 toxicity studies are presented in Table 4.5-2. These values are based on the minimum TL_{50} value obtained during our acute bioassays (see Table 4.2-1) and an application factor of 0.05. This application factor has been recommended in the 1973 Water Quality Criteria (EPA "Blue Book") for materials that are not persistent (i.e., having a half life of less than 4 days) and not having accumulative effects. Values in Table 4.5-1 are for each compound individually in the aquatic environment; synergistic effects are not considered, as yet, in our recommendations.

A comparison between the estimated safe concentrations for the phenolic compounds in Table 4.5-2 to that in Table 4.5-1 for total phenolics reveals that the suggested safe value for total phenolics (1 $\mu\text{g/l}$) is considerably below the estimated safe level for any phenolic compound individually (0.1 - 5.0 mg/l). As has been pointed out in our quarterly report for 5 June to 5 September 1977, the value for total phenolics is based primarily on the E.P.A.'s "Red Book" criterion, which includes chlorophenolics. Inclusion of the chlorophenolics in this group has resulted in a substantial depression of the criterion due to the intense organoleptic properties of the chlorophenolics. While we do not agree with this grouping of phenolics, we have tentatively adopted the Red Book's value until we have obtained further data for phenolic compounds, both from the literature and from our toxicity studies.

All estimated safe concentrations that we report during the project (e.g., Tables 4.5-1 and 4.5-2) are intended only as preliminary performance criteria for proposed process water treatment systems. Final performance criteria recommendations will be presented in our completion report at the end of Year 3.

Table 4.5-1. Summary of tentative recommendations for estimated safe concentrations (ESC) of chemicals in aquatic environments.^a

Chemical	ESC µg/l
Arsenic	50
Cadmium	0.1 (soft water) 1.2 (hard water)
Chromium	20
Cobalt	50
Copper	4
Manganese	100
Nickel	30
Selenium	10
Zinc	1 (soft water) 10 (hard water)
Ammonia	20 (as NH ₃)
Cyanide	5 (as CN ⁻)
Hydrogen sulfide	2 (as H ₂ S)
Total phenolics (includes chlorophenols)	1 ^b

^aFinal recommendations will be made at the completion of this project

^bSee text for discussion.

Table 4.5-2. Tentative estimated safe concentrations for selected single organics in aquatic environments based on minimum TL₅₀ values from our model compound toxicity studies and an application factor of 0.05.^a

Compound	Minimum TL ₅₀ (mg/l)	Tentative ESC (mg/l)
Phenol	8.9	0.5
Catechol	3.5	0.2
Resorcinol	~100	5.0
o-Cresol	8.4	0.4
m-Cresol	8.9	0.4
p-Cresol	8.6	0.4
Naphthalene	2.2	0.1

^aSee text for discussion.

5.0 ADDITIONAL ACTIVITIES

5.1 Visitors Received

On 17 November, Dr. John Cairns, Jr., from VPI-SU in Blacksburg, Virginia, spent the day with project staff and visited the Laramie Energy Research Center. Fruitful discussions with Dr. Cairns will assist the project, especially with behavioral and physiological monitoring of toxicant exposed fish.

On 7-8 December, Mr. Larry Brooke from the University of Wisconsin-Superior visited project personnel and discussed aspects of toxicity and bioaccumulation of organics in fish as related to an EPA sponsored project with which he is associated.

5.2 Response to Inquiries

In October, Dr. Steve Herbes, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, called to inquire about the overall scope and objectives of our research. This information was provided by Bergman. Herbes' intent was to use the information in a paper overviewing ongoing environmental research on fossil fuels at a recent meeting at Battelle Northwest in Richland, Washington.

5.3 Meetings Attended

American Fisheries Society, Annual Meeting, Vancouver, B.C., September 14-17, 1977

-Bergman, DeGraeve, R. Johnson, Fannin

Workshop on Organic and Inorganic Chemicals Associated with Energy, Environmental Research Lab - Duluth, Minnesota, September 26-27, 1977

-Bergman

American Society of Testing and materials, Cleveland, Ohio, October 31-November 1, 1977

-Anderson

5.4 Papers Submitted or Presented

5.5.1 Papers Submitted

"Effects of oil shale retort water on rainbow trout." A. D. Anderson, M. E. Lebsack, D. S. Ferrier and R. E. Poulson. Submitted for American Chemical Society Annual Meeting, March 12-17, 1978 (Copy of abstract shown as Appendix B).

5.5.2 Papers Presented

"Environmental screening of aquatic contaminants from in situ fossil fuel processing." Harold Bergman. Rocky Mountain Institute of Energy and Environment, Industrial Fund Advisory Meeting, November 4, 1977.

5.5 Publications

5.5.1 Papers in Preparation

Toxicity of in situ coal gasification process water and major phenolic constituents to fathead minnow, rainbow trout and Daphnia pulex (tentative title)--Lead author: DeGraeve.

Bibliography of aquatic ecosystem effects of organic constituents found in process waters from advanced fossil fuel processing technologies (tentative title)--Lead author: Marcus.

A noise-free method for separately recording opercular and heart activity from free-swimming fish. M.J. Yakimovich and H.L. Bergman.

5.5.2 Papers Submitted

None

5.5.3 Papers in Press

None

5.5.4 Papers Published

None

5.6 Facilities

During this reporting period we purchased, set up and began routinely using a Waters high performance liquid chromatograph. The use of the HPLC will allow us to meet project objectives, especially with degradation and bioaccumulation tasks, which have slipped since the beginning of the project because of inadequate analytical equipment. Certain improvements in Bergman's lab have been completed, primarily to accomodate increased organic extraction and analysis work now possible with the HPLC.

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7.0 APPENDICES

- A. Chemical Analyses of Test Waters
- B. Effects of Oil Shale Retort Water on Rainbow Trout. A. D. Anderson, M. E. Lebsack, D. S. Ferrier and R. E. Poulson (Abstract submitted for American Chemical Society Annual Meeting, March 12-17, 1978).

APPENDIX A

Chemical Analyses of Test Waters

Inorganic Analyses

Standard methods (APHA et al. 1975) were used to monitor water quality for all acute and chronic toxicity tests. Dissolved oxygen (Azide Modification #422 B) was measured daily for acute tests and every other day for chronic tests. Alkalinity (#403) and hardness (EDTA Titrimetric Method #309 B) titrations were performed once a week for both acute and chronic tests.

Conductivity and pH were measured once a week with a Hach Conductivity Meter and a Corning Model 12 pH meter, respectively. Free CO₂ was calculated from alkalinity and pH values by the method of Rainwater and Thatcher (1959).

Organic Analyses

Toxicant concentrations in acute and chronic tests and degradation experiments were monitored chromatographically. The areas under all peaks in the gas chromatographic runs were measured and compared to the areas of injected standards; however, the peaks eluting from the liquid chromatographic runs were symmetrical and reproducible enough to allow comparisons of peak heights to determine concentrations.

A Varian Aerograph Model 600-D gas chromatograph (GC) equipped with a flame ionization detector was used to determine single-compound toxicant concentrations before the liquid chromatograph was purchased. Table A-1 presents the compounds analyzed and the operating conditions and columns used on the GC.

The Waters Associates Model 204 ALC/GPC high performance liquid chromatograph (HPLC) equipped with a Model 660 Solvent Programmer and Model 440 UV Absorbance Detector was set up in early November. Single compound analyses were performed with Burdick and Jackson solvents at the isocratic conditions listed in Table A-2.

The Hanna 3 process water used in the chronic embryo-larval bioassay was analyzed by a gradient elution technique. Linear solvent programs were run from 0-100% CH₃CN/H₂O over 10 minutes at 2.0 ml/min total solvent flow rate on a 3.9-mm ID x 30-cm long reverse-phase μ -C₁₈ column that allows the compounds in the process water to elute in order of decreasing polarity. We chose those conditions after comparing several gradient parameters (see Figures A-1 and A-2) with varying degrees of resolution and total chromatographing time. The 20 minute linear gradient at 2.0 ml/min provided the best resolution, but that advantage was overshadowed by the extra 10 minutes required for a return to baseline. The program conditions we chose required 16 minutes to elute the samples and 10 minutes to return to initial conditions with a stable baseline.

Figure A-3 presents chromatograms of a set of standards at two different attenuations that demonstrate the gradient process. Resorcinol, phenol, benzene, toluene, naphthalene, phenanthrene, pyrene and benzo(a)pyrene elute by decreasing polarity with characteristic retention times that can be compared

Table A-1. Gas chromatographic conditions for organic analyses of water.^a

Compound	Injector Temperature (°C)	Column Temperature (°C)	Column
Phenol	215	175	2 mm ID x 4 ft. long glass column; 20% Carbowax 20M on 80/100 mesh Chromosorb W-HP, AW-DMCS
o-cresol	215	175	"
m-cresol	215	175	"
p-cresol	215	175	"
Naphthalene	255	100	2 mm ID x 6 ft. long glass column; 5% OV-1 on 80/100 mesh Chromosorb W-HP, AW-DMCS
Phenanthrene	255	175	"
Pyrene	255	200	"

^aVarian Model 600-D GC with flame ionization detector and gas flow rates for air = 400 ml/min at 10 psi; H₂ = 27 ml/min at 10 psi; and N₂ = 28 ml/min at 25 psi.

Table A-2. Liquid chromatographic conditions for organic analyses of water.^a

Compound	Solvent Ratio	Retention Time (min)
<u>Single Compound Analyses</u>		
Phenol	60% CH ₃ CN/H ₂ O	2.0
o-cresol	60% MeOH/H ₂ O	3.0
o-cresol	60% CH ₃ CN/H ₂ O	2.3
Benzene	80% MeOH/H ₂ O	2.3
Benzene	80% CH ₃ CN/H ₂ O	2.1
Naphthalene	80% MeOH/H ₂ O	3.0
<u>Multiple Standard Analyses</u>		
Resorcinol	0-100% CH ₃ CN/H ₂ O	6.1
Phenol	linear gradient program	7.7
Benzene	10 min. at 2.0	9.7
Toluene	ml/min	10.5
Naphthalene		11.0
Phenanthrene		11.8
Pyrene		12.3
Benzo(a)pyrene		13.2

^aWaters Associates Model 204 ALC/GPC with Model 660 Solvent Programmer, Model 440 UV Absorbance Detector (254 nm), Model U6K 2 ml Injector, 3.9 mm ID x 30 cm μ -C₁₈ reverse-phase column, and 2.0 ml/min solvent flow rates.

10 μ l 1:2 Hanna 3 dilution

0-100% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$; 0.1 AUFS

10 min linear gradient @ 2.0 ml/min

10 μ l 1:2 Hanna 3 dilution

0-100% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$; 0.1 AUFS

10 min linear gradient @ 1.0 ml/min

Figure A-1. Trial HPLC gradient elution chromatograms of Hanna 3 condenser water.

10 μ l 1:2 Hanna 3 dilution

0-100% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$; 0.1 AUFS

20 min linear gradient @ 2.0 ml/min

Figure A-2. Trial HPLC gradient elution chromatogram of Hanna 3 condenser water.

Figure A-3. HPLC chromatograms of standards.

to chromatograms of the process water. Excellent baseline resolution is achieved between all the standards and only minimal baselines are contributed by the carrier solvents. The peaks eluting at 5.0-5.5 minutes indicate polar aromatics not removed from the carrier water by our present cleaning techniques.

Figure A-4 shows the raw process water chromatographed at 1.0 AUFS and 0.1 AUFS. Two large peaks dominate the lower sensitivity chromatogram at 7:45 and 8:40 minutes; we are tentatively identifying the 7:45 peak as phenol at about 2,300 mg/l. The 0.1 AUFS chromatogram provides better sensitivity with more peaks that we only intend to classify in general ranges based on the retention times of the injected standards:

- < 4.5 min - straight-chain acids and ketones
- 4.5 - 8.0 min - polar aromatic acids and phenols
- 8.0 - 11.0 min - single-ring, non-polar aromatics
- 11.0 - 14.0 min - polynuclear aromatics (PNA's)

The majority of these peaks appear in the polar eluting region. The raised baseline in the non-polar PNA region indicates the presence of this class of compounds, though the lack of distinct peaks must be attributed to their low concentrations compared to those in the phenolic range. Long gradients at high sensitivity would be required to resolve the individual compounds in this non-polar region.

Figure A-5 is a chromatogram of Tank A (highest process water concentration) in the chronic embryo-larval test. We decided to use the major peak at 7:45 to quantify the dilutions of the process water in each aquarium, as it is the only measurable peak in the middle and lower concentration tanks, even with the 1 ml injections and 0.01-0.05 AUFS sensitivity that are required for these extreme dilutions (less than 1/1000). The embryo-larval bioassay has not been completed and the results will not be reported until the first quarter of 1978.

The reproducibility of this HPLC technique was demonstrated by measuring peaks 7:45 and 8:40 on 10 consecutive solvent programs of a 0.1% process water dilution at 1 ml injections and 0.1 AUFS. For peak 7:45 the heights ranged from 85.0 to 87.0 blocks with a standard deviation of 1.0%; peak 8:40 displayed a range of 28.0-28.5 blocks and a standard deviation of 0.8%. Thus we are confident our chromatograms are reproducible and accurate even in the solvent programming mode.

10 μ l Hanna 3 water

0-100% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$; 1.0 AUFS

10 min linear gradient @ 2.0 ml/min

10 μ l Hanna 3 water

0-100% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$; 0.1 AUFS

10 min linear gradient @ 2.0 ml/min

Figure A-4. HPLC chromatograms of Hanna 3 condenser water.

Figure A-5. HPLC chromatogram of embryo-larval bioassay water (Tank A).

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EFFECTS OF AN OIL SHALE RETORT WATER ON RAINBOW TROUT

A.D. Anderson, M.E. Lebsack, D.S. Farrier¹ and R.E. Poulson¹

School of Pharmacy, University of Wyoming, Laramie, Wyoming 82071
and

¹Laramie Energy Research Center
U.S. Department of Energy, Laramie, Wyoming 82070

In light of the imminent need for the recovery of commercial quantities of petroleum from oil shale deposits, identifying the potential ecologic and economic effects of in situ oil shale processing is imperative. Attendant with the processing of oil shale deposits is the copious production of by-product water (retort water¹). The potential for ecological contact with this water, either from surface disposal or through underground migration has not been defined. However, some potential for contact with aquatic systems may exist and, therefore, investigations were undertaken to determine the potential magnitude of the effects on aquatic species. Identification of behavioral and physiological changes in rainbow trout after exposure to oil shale retort water may provide bioassay systems for evaluating the efficacy of waste water control systems.

Unfed 11-18cm rainbow trout were used to determine the flow-through 96hr toxicity of retort water exposure. The 95% confidence limits for the LC50 were 3.79 and 3.91ml/L as determined by a binomial test.

Investigations of several physiological parameters, serum electrolytes, packed cell volume, hemoglobin, creatinine phosphokinase, glutamic-oxalacetic transaminase, lactate dehydrogenase, glutamic-pyruvic transaminase, acid phosphatase and alkaline phosphatase (AP), in trout exposed to retort water showed detectable differences between controls and exposed fish for only AP. AP levels were inversely related to increasing length of exposure retort water (Table 1). Increasing concentrations of retort water also decreased AP activity (Tables 2,3). Retort water also inhibits trout liver AP activity in vitro. Studies are presently in progress to measure AP levels in different organs in treated and control fish to determine the origin of these decreased serum AP levels.

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¹Retort water used in this study was obtained from the Laramie Energy Research Center experimental in situ oil shale retorting facility located near Rock Springs, Wyoming and carries the designation "OMEGA-9 water". The retort water was filtered (0.4 microns) to remove suspended material and stored at 4°C. Chemical parameters indicative of the composition of this water are: $\text{NH}_4^+ = 4838\text{mg/L}$, $\text{S}_2\text{O}_3 = 2360\text{mg/L}$, $\text{HCO}_3^- = 15,100\text{mg/L}$, pH 8.5, total organic carbon = 1,020mg/L and total dissolved solids = 13,900mg/L.