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

By
Harold L. Bergman

December 1978

Work Performed Under Contract No. ET-78-C-03-1761

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

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

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The Rocky Mountain Institute of Energy and Environment
The University of Wyoming

Submitted by

Harold L. Bergman
Principal Investigator

Department of Zoology and Physiology
The University of Wyoming
Laramie, Wyoming
82071

31 December 1978

CONTENTS

1.0 SUMMARY

This is the second 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. During 1978, the project was organized into five tasks: (1) literature review, (2) toxicity studies, (3) degradation studies, (4) bioaccumulation studies, and (5) recommendations. During the 1979 calendar year, the final year of the originally proposed project, work will be reoriented slightly into four project tasks: (1) literature review updates, (2) process water screening, (3) methods development, and (4) recommendations. In the process water screening task we anticipate studying ten process waters.

Accomplishments in the five project tasks for 1978 are summarized as follows:

Task 1: Literature Review

-A bibliographic search was completed covering 1970 to 1978 citations in six computerized databases to identify published literature on the aquatic effects of and treatment technologies for over 470 organic compounds that have been identified in process waters from advanced fossil-fuel technologies.

-We compiled and cross-indexed 1,314 relevant citations obtained from the search in preparation for publication of a bibliography for use by other researchers.

-We continued updating computerized searches through 1978 citations and compiling literature to prepare for publication of a bibliography update as an appendix to our final report.

Task 2: Toxicity Studies

-In flow-through bioassays with and analyses of Omega-9 oil shale retort water, we determined that the principal constituents contributing to acute toxicity were inorganics with ammonia possibly being the most detrimental component; 96-hr TL_{50} dilutions for Omega-9 were 0.57% for fathead minnows, 0.42 and 0.51% for rainbow trout in two independent tests, and 0.54% for Daphnia pulex; in 69-day embryo-larval bioassays with rainbow trout, threshold growth effects occurred at 0.16% Omega-9 retort water.

-Geokinetics-9 oil shale retort water displayed acute toxicities similar to Omega-9 water; 96-hr TL_{50} values for Geokinetics water were 0.88% for fathead minnows, 0.46% for rainbow trout and 0.56% for Daphnia pulex.

-With Hanna-3 UCG condenser water we determined that the principal constituents contributing to acute toxicity were phenolic compounds and ammonia, with ammonia possibly the most detrimental component; 96-hr TL_{50} dilutions for Hanna-3 water were 0.11% for fathead minnows and 0.10% for rainbow trout; in 30-day embryo-larval bioassays with fathead minnows, threshold effects on fry survival occurred at 0.01% Hanna-3 condenser water.

-In two completed 30-day fathead-minnow embryo-larval bioassays, threshold effects on fry growth occurred at 2.5 mg/ℓ phenol and threshold egg-hatchability and fry-growth effects occurred at 0.85 mg/ℓ naphthalene.

-Flow-through, 96-hr bioassays on seven constituents of advanced fossil-fuel process waters resulted in TL_{50} values (mg/ℓ) for rainbow trout/fathead minnows as follows: benzene (5.3/>15.1); benzonitrile (32/64); p-benzoquinone (0.12/0.04); hydroquinone (0.04/0.02); indene (8.15/18.2); naphthalene (8.95 mg/ℓ for fathead minnows at 25°C compared to 4.9 mg/ℓ determined last year at 14°C); phenol (24.9 mg/ℓ for fathead minnows at 25°C compared to 67.5 mg/ℓ determined last year at 14°C).

-Fathead-minnow fry in 48-hr static bioassays with two process waters and seven constituents had TL_{50} values of 0.32% Geokinetics -9 oil shale retort water; 7.6% RS-12 pre-retort ground water, 142 mg/ℓ aniline, 163 mg/ℓ benzonitrile, 0.02 mg/ℓ p-benzoquinone, >25 mg/ℓ indene, 225 mg/ℓ pyridine (72-hr), 197 mg/ℓ pyrrole and 71 mg/ℓ thiophene.

Task 3: Degradation Studies

-A microbial shake-flask test system was developed for comparing potential environmental persistence of organic compounds, and we thoroughly evaluated the effect of six different parameters on the degradability of phenol.

-Using the shake-flask degradation system and bacterial inocula from an oil refinery settling pond, phenol completely degraded by 96 hr, benzene degraded to <10% by 192 hr, and naphthalene completely degraded by 48 hr; using bacterial inocula from a eutrophic lake, phenol degraded by 192 hr, while benzene and naphthalene were not affected by 240 hr.

-In 60-day microbial degradation studies of Omega-9 oil shale retort water using the shake-flask system inoculated with bacteria from an oil refinery settling pond, HPLC analyses indicated that: (1) all of the major organic compounds in the Omega-9 water have relative polarities equal to or greater than naphthalene; (2) most peaks initially present decreased over time; and (3) as original peaks decreased in height new peaks often simultaneously appeared in the more polar region of the chromatograms.

Task 4: Bioaccumulation Studies

-Exhaustive steam distillation with n-octanol of fathead minnows exposed to 14C-phenol provided extraction efficiencies of 80%; similar extraction of 14C-phenol in water provided extraction efficiencies of 88%.

-Extraction of 14C-phenol-exposed fish using a routine $CHCl_3$/Na_2SO_4 homogenization-extraction procedure produced extraction efficiencies of only 9%.

-Use of n-octanol as an extraction solvent in steam distillation is unsuitable for HPLC analysis because of excessive peak spreading and decreased resolution; preliminary experiments suggest that n-butyl ether produces somewhat lower extraction efficiencies but is more compatible with HPLC analysis.

Task 5: Recommendations

-Based on our embryo-larval toxicity results we recommend maximum acceptable toxicant concentrations (MATC) of 0.08% Omega-9 oil shale retort water, 0.003% Hanna-3 UCG condenser water, 0.75 mg/ℓ phenol and 0.45 mg/ℓ naphthalene.

-Based on flow-through, 96-hr bioassays and application factors estimated from similar process waters or constituents we recommend estimated safe concentrations (ESC) of 0.09% Geokinetics-9 oil shale retort water, 0.3 mg/ℓ benzene, 0.2 mg/ℓ catechol, 5.0 mg/ℓ resorcinol, 0.001 mg/ℓ hydroquinone, 0.4 mg/ℓ o-cresol, 0.4 mg/ℓ m-cresol, 0.4 mg/ℓ p-cresol, 1.2 mg/ℓ benzonitrile, 0.002 mg/ℓ p-benzoquinone, and 0.4 mg/ℓ indene; all ESC values are based on single toxicant exposure and do not consider possible synergistic toxicities.

2.0 INTRODUCTION

This is the second annual progress report issued for the Year 2 funding period under Task Agreement #1, DOE-UWRMIEE Contract #DE-AS20-79 LC 01761, a project to evaluate the effects of aqueous effluents from in situ fossil fuel processing technologies on aquatic biota. The 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 Technology Center.

Briefly, the goals of the project are:

1. Evaluate the toxicity of process water effluents on aquatic biota;

2. Recommend maximum exposure concentrations for process water constituents; and

3. Assist DOE in using project data and recommendations to design control technologies and to assess environmental impacts.

The project objectives for Year 2 were pursued through five tasks:

1. Literature reviews on process water constituents, possible environmental impacts and potential control technologies;

2. Toxicity bioassays on the effects of process waters and six process water constituents on aquatic biota;

3. Biodegradation studies on process water constituents;

4. Bioaccumulation factor estimation for the compounds tested in the toxicity bioassays; 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, progress toward completion of the project goals is presented in sections on Administration, Task Reports, and Additional Activities.

3.0 ADMINISTRATION

3.1 Staff

The following individuals worked part or full-time on the various project tasks during the past year (see Section 4.0 TASK REPORTS for task assignments).

-Harold L. Bergman (Principal Investigator)
-A. Duane Anderson (Co-Investigator)
-Michael Parker (Co-Investigator)
-G. Michael DeGraeve (Research Associate)
-M. E. Lebsack (Research Associate)
-Timothy Fannin (Research Assistant)
-Dianne Geiger (Research Assistant)
-Rodney Johnson (Research Assistant)
-Michael Marcus (Scientist I)
-Joseph Meyer (Scientist I)
-Joan Anderson (Technician)
-Carolyn Larusso (Technician)
-Ron Overcast (Technician)
-Dennis Woods (Technician)
-Linda Zeveloff (Technician)

3.2 Staff Meetings and Seminars

During the past year we continued to hold periodic staff meetings to review progress, present results and conclusions for staff criticism, and plan up-coming work.

A project review meeting was held in September with project officers Dr. Ken Biesinger, EPA-Duluth, and Dr. David Farrier, DOE-Laramie, present.

3.3 Administrative Problems

Early in the up-coming year the main administrative problems will involve formal agreement on the Year 3 work plan and contract. We anticipate that the work plan will be revised to cover four major tasks: (1) Literature review updates; (2) Process water screening; (3) Methods development; and (4) Recommendations.

4.0 TASK REPORTS

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

4.1.1 Work Completed This Year

We compiled a list of over 470 organic compounds which have been identified as constituents of fossil fuels and process waters from advanced fossil-fuel processing technologies. References used to compile this list include Pellizzari (1978), Gulf South Research Institute (1977a,b; 1978), Barbour et al. (1978), Dark et al. (1977), Hills et al. (1968), Schmidt et al. (1974), Schmidt-Collerus (1974, 1976), King et al. (1975), and Denver Research Institute and Environmental Engineering Division (1977). In addition, we consulted unpublished lists of organics in fossil-fuel process wastes. The list of compounds was then expanded to include synonyms for each compound (Christensen and Luginbyhl 1975, Weast 1977, Windholz et al. 1976, Verschueren 1977).Nearly 3000 chemical names are included in the final list.

Based on our finalized list we conducted a computer search of six bibliography data bases in conjunction with the University of Wyoming Science and Technology Library and the Bibliographic Retrieval Service, Inc., Scotia, New York. The bases searched (Table 4.1-1) include BIOSIS PREVIEWS (Biological Abstracts and BioResearch Index), CA CONDENSATES (Chemical Abstracts), MEDLARS (National Library of Medicine: Index Medicus), POLLUTION (Pollution Abstracts), CDI (Comprehensive Dissertation Abstracts), and NTIS (National Technical Information Service Abstracts). The strategy used for the searches involved crossing each compound and its synonyms with appropriate key words (Table 4.1-2) to identify available literature pertaining to toxicity, degradation, bioaccumulation, cycling and structure-activity of each compound. The search resulted in 12,609 citations (Table 4.1-3). This output was recorded on computer tape for final sorting through the facilities of the University of Wyoming Computer Center.

All citations obtained from the search of the six bibliographic data bases were sorted for relevance to our bibliography on the aquatic ecosystem effects of organic compounds associated with advanced fossil fuel processing technologies. Of all citations obtained, only 1,829 were retained as relevant (Table 4.1-3). The citations retained were combined into a single alphabetical list to remove duplicates, leaving 1,314 citations for publication in our final indexed bibliography.

Permission has been obtained from Dissertation Abstracts, National Technical Information Service and Pollution Abstracts to reproduce citations obtained from these data bases. The legal advisor of the National Institute of Health advised us that since the information we intend to use from the Medlars database has been generated by the Government and therefore not subject to copyright under the Federal Copyright Act, we do not need permission to use it. And, while preliminary arrangements have been made to obtain permission to use material from Biological Abstracts and Chemical Abstracts, final arrangements await a decision on the number of copies of the bibliography to be printed and the length of time the bibliography will be available for distribution.

Table 4.1-1. Issues and dates of hard copy indexes corresponding to the data base material searched for the bibliography.

Index	Initial			Final		
	Vol.	No.	Date	Vol.	No.	Date
Biological Abstracts	51	1	January 1, 1970	65	8	April 15, 1978
BioResearch Index	6	1	January, 1970	14	4	April, 1978
Chemical Abstracts	72	1	January 5, 1970	87	26	December 26, 1977
Dissertation Abstracts	20	7	January, 1960	38	10	April, 1978
Medlars	10	-	January, 1969	19	5	May, 1978
NTIS[a]	70	1	January 1, 1970	78	8	April 14, 1978
Pollution Abstracts	1	1	May, 1970	8	6	November, 1977

[a]Equivalent hard copy is "Government Reports Announcements and Index."

Table 4.1-2. The two key-word lists crossed with the results of the chemical searches to obtain the initial set of citations included in this bibliography ($ is a truncation device to include in the search all words beginning as indicated to the $. Numbers following the $ limit the number of words searched only to those containing the number of letters indicated beyond the truncation. Point, e.g., "LAKE$1" includes "LAKES" but not "LAKEWOOD.").

List 1	List 2
CHROMATOGRAPH$5	TOXIC$11
EXTRACT$9	BIOASSAY$1
IDENTIF$9	POLLUT$4
ANALY$7	AVOID$5
SEPARAT$7	TOLERA$4
DIGEST$9	ACUTE$
STRUCTURE	CHRONIC$
ACTIVITY	ACCLIMAT$8
PARTITION	LETHAL$3
COEFFICIENT	MORTAL$3
	SURVIV$7
FISH	GROWTH
FISHES	REPRODUC$7
TROUT$1	
MINNOW$1	ENZYM$8
INVERTEBRAT$1	PHYSIOLOG$6
LARVA$1	PATHOLOG$6
DAPHNIA$	HISTOPATHOLOG$6
WATER	HISTOLOG$6
FLEA$1	DEGRAD$7
CLADOCERA$	OXIDAT$13
COPEPOD$	BIOACCUMULAT$4
ROTIFER$	BIOMAGNIFACT$4
	BIOCONCENTRAT$4
AQUATIC INSECTS	
AQUATIC ANIMALS	ACCUMULAT$4
AQUATIC LIFE	MAGNIFICAT$4
PHYTOPLANKT$4	CONCENTRAT$4
ZOOPLANKT$4	METABOL$7
PLANKT$4	NEUROLOG$6
ALGA$	EMBRYO$8
BACTERIA$2	CYCLE
MICROB$3	CYCLES
MICROORGANISM$	CYCLING
AQUATIC$1	
LAKE$1	
POND$1	
POOL$1	
STREAM$1	
BROOK$1	
CREEK$1	
RIVER$1	
TRIBUTAR$3	
FRESHWATER$1	
WATER$1	
ECOSYSTEM$	
MICROCOSM$	
EFFLUENT$	
AQUEOUS	

Table 4.1-3. Number of citations obtained and retained from the bibliographic data bases with the percentage of citations retained from the bases.

Bibliographic Data Base	Obtained	Retained	Percent
Biosis Previews	2,780	486	17.5
Chem. Abst. Condensates	4,116	892	21.7
Medlars	3,043	161	5.3
Pollution Abst.	481	109	22.7
Comp. Diss. Abst.	551	28	5.1
NTIS	1,638	153	9.3
Total	12,609	1,829[a]	14.5[a]

[a]Includes duplicate citations; after removal of duplicates, 1,314 citations remained accounting for 10.4 percent of the original citations obtained.

The unexpectedly low number of relevant citations found for the 470 organic compounds (over 3000 chemical names including synonyms) reflects the scarcity of published information on these compounds in aquatic ecosystems. Also many of the 1,314 citations retained dealt with phenolic compounds or cyanide, with few or no citations obtained for the large majority of chemical names searched. Thus, when the bibliography is published early next year much of its value to other investigators will be to confirm the absence of literature for certain compounds of interest.

4.1.2 Work in Progress

Computer programs were written to index the finalized citation list by authors, by chemicals and by key words (e.g. toxicity, biodegradation, structure-activity, etc.). In addition, a program was written to print the bibliographic pages. Problems with debugging the indexing programs prevented the completion of the bibliography this year. However, we expect these problems to be resolved and the bibliography submitted for publication within a month.

Expansion of our reference collection pertaining to all phases of our studies is continuing. Monthly updates of our searches of the six computerized databases are being indexed and will be appended to our Final Report at the end of Contract Year 3.

4.1.3 Work to be Performed Next Year

We expect to complete the bibliography and submit it for publication in January. Monthly database search updates will be compiled and sorted by subject for appending to the final report at the end of Year 3. We expect this update to contain approximately 400 additional citations.

4.2 Task 2: Toxicity Studies (DeGraeve, Lebsack, Geiger, Woods, Overcast, Johnson, Meyer, A. D. Anderson, Bergman)

4.2.1 Work Completed This Year

During 1978 we completed the following: (1) flow-through 96-hr bioassays exposing fathead minnows (Pimephales promelas) and rainbow trout (Salmo gairdneri) to Hanna-3 underground coal gasification (UCG) condenser water, Omega-9 oil shale retort water, Geokinetics-9 oil shale retort water, the major inorganic constituents of Omega-9 retort water, benzene, benzonitrile, p-benzoquinone, hydroquinone, indene, naphthalene and phenol; (2) flow-through 48-hr bioassays exposing Daphnia pulex to Omega-9 oil shale retort water, Geokinetics-9 oil shale retort water, benzene, hydroquinone and indene; (3) static acute tests exposing juvenile fathead minnows to Hanna-4A UCG condenser water, Geokinetics-9 oil shale retort water, RS-12 pre-retort ground water, aniline, benzonitrile, p-benzoquinone, indene, pyridine, pyrrole and thiophene; (4) flow-through embryo-larval bioassays exposing fathead minnow eggs and fry to Hanna-3 UCG condenser water, naphthalene and phenol (a fourth embryo-larval bioassay with fathead minnows was begun with p-benzoquinone but was terminated because of rapid degradation of the test compound in our flow-through diluter); (5) flow-through embryo-larval bioassays exposing rainbow trout eggs and fry to Omega-9 oil shale retort water (a similar test with phenol is underway); (6) biochemical parameter evaluation in rainbow trout exposed to sublethal concentrations of Omega-9 oil shale retort water and an aritificàl mixture of major inorganic constituents in Omega-9 water; (7) gill histopathology evaluations of rainbow trout exposed to Hanna-3 UCG condenser water and phenol; and (8) tests of an apparatus for measuring the avoidance response of fish to pollutants with one evaluation of the avoidance of sublethal concentrations of phenol by rainbow trout.

Methods Synopsis

Flow-through acute bioassays were conducted according to standard toxicity testing procedures as outlined by the U.S. Environmental Protection Agency (1974). Test organisms were exposed to seven concentrations of the test solution plus well water controls. Fish were tested for 96 hours in 28-ℓ (rainbow trout) and 14-ℓ (fathead minnows) aquaria, while Daphnia pulex were exposed for 48 hours in test chambers as described by DeGraeve et al. (1977). In the 96-hr bioassays with an artificial mixture of major inorganic constituents of Omega-9 water, the constituents shown in Table 4.2-1 were mixed to produce a starting solution equal in concentration to the Omega-9 water followed by serial dilution of this mixture as described above. For static bioassays, fish were exposed to well water-diluted toxicants for 48 hours in 2-ℓ beakers. These tests were done primarily to determine the general ranges of toxicities of the process waters or single compounds so that we could decide if they warranted flow-through tests.

Embryo-larval tests were conducted according to procedures outlined by McKim (1977); fathead minnow eggs and fry were exposed to seven concentrations of the toxicants through 30 days of age. Egg survival and hatchability plus fry survival and growth were monitored. For the rainbow trout embryo-larval bioassay with Omega-9 oil shale retort water, fertilized eggs were exposed in five dilutions of the Omega-9 water through hatching and an initial period of fry growth.

Table 4.2-1. Principal inorganic constituents in Omega-9 oil shale retort water used in an artificial mixture to determine acute toxicity of inorganic constituents in Omega-9 water.[a]

Constituent	Concentration (mg/ℓ)
HCO_3^-	15,940
Na^+	4,333
NH_4^+	3,470
$S_2O_3^{2-}$	2,740
SO_4^{3-}	1,990
Cl^-	824
CO_3^{2-}	500
$S_4O_6^{2-}$	280
SCN^-	123
F^-	60
K^+	47
Mg^{2+}	20
Ca^{2+}	12

[a]Source: Personal Communication, D. S. Farrier, U.S. Department of Energy, Laramie Energy Technology Center, Laramie, WY.

Water quality of test waters during all of the acute and embryo-larval bioassays is summarized in Table 4.2-2. Well water was used in all bioassays except that dechlorinated tap water was used during the rainbow trout, embryo-larval test with Omega-9 oil shale retort water.

Biochemical-level tests were conducted on juvenile rainbow trout exposed to sublethal concentrations of Omega-9 water (0.075 to 0.31%) and to sublethal concentrations (0.4%) of an artificial mixture of major inorganic constituents found in Omega-9 water (Table 4.2-1). In the Omega-9 studies, livers from some rainbow trout were removed after a 96-hr exposure for enzyme assays or determination of the concentration of 22 elements; the hepatic enzyme activities determined were glutamate-pyruvate transaminase, glutamate dehydrogenase and alkaline phosphatase. Also, blood was sampled from these fish for determination of packed cell volume, hemoglobin concentration, plasma alkaline phosphatase activity, plasma protein, and plasma ammonia. Blood obtained from rainbow trout exposed to a 0.4% solution of the major inorganic constituents from Omega-9 water was measured for packed cell volume, plasma alkaline phosphatase activity, plasma protein, and plasma ammonia.

Gill histopathology was evaluated in juvenile rainbow trout exposed to seven sublethal concentrations of phenol for 4, 14 and 21 days and trout surviving the 96-hr acute bioassay exposure to Hanna-3 UCG condenser water. After each exposure period gill filaments were removed, fixed in glutaraldehyde, embedded in plastic, and sectioned for investigation with light and electron microscopy.

Toxicant avoidance tests were initiated by placing one rainbow trout in each of four avoidance tanks supplied by a modified proportional diluter. Concurrently, we started an overhead-mounted camera which took a picture of the tanks every 10 minutes. Twenty-four hours later we began the toxicant flow to three of the four tanks; the fourth tank was maintained as a double-well-water control. After 48 hr of toxicant flow the lines from the diluter were switched, thus causing the toxicant and well water flows to switch locations in the avoidance tanks. After 96 hr of toxicant flow the experiment was terminated.

Results

The results of acute flow-through bioassays are presented in Table 4.2-3, and Table 4.2-4 gives the results of all static acute tests completed in 1978. The 96-hr (fathead minnow and rainbow trout) and 48-hr (*Daphnia pulex*) TL_{50} dilutions for all three process waters tested were at dilutions of less than 1% for all test animals, with the Hanna-3 UCG TL_{50} dilution at about 0.1%, the Omega-9 retort water TL_{50} dilution at 0.4 to 0.5%, and the Geokinetics -9 retort water TL_{50} dilution at about 0.4 to 0.9% (Table 4.2-3). The 96-hr TL_{50} dilution for the major inorganic constituents of Omega-9 retort water, when tested in a mixture at concentrations similar to those in Omega-9 water, was 0.56% for rainbow trout and 1.12% for fathead minnows. Of the seven individual compounds tested in acute flow-through bioassays (Table 4.2-3) and the four additional compounds tested in acute static bioassays (Table 4.2-4), the most toxic were p-benzoquinone and hydroquinone with TL_{50} concentrations all below 1 mg/ℓ for the three species of test animals.

Table 4.2-2. Summary of water quality of test waters during bioassays for process waters and individual constituents; means (range).

Water quality parameter	Bioassays using well water dilution[a]		Bioassays using dechlorinated tap water dilution[b]	
	Control tanks	Highest concentration toxicant tanks	Control tanks	Highest concentration toxicant tanks
Dissolved oxygen (mg/ℓ)	7.1 (4.6-8.3)	6.4 (3.2-8.5)	8.8 (8.0-9.7)	8.1 (7.5-8.9)
pH (units)	8.0 (7.8-8.2)	8.1 (7.7-8.5)	7.5 (7.4-7.7)	7.8 (7.7-7.9)
Alkalinity (mg/ℓ as $CaCO_3$)	151 (144-155)	179 (143-282)	166 (161-173)	201 (189-208)
Hardness (mg/ℓ as $CaCO_3$)	632 (569-742)	631 (551-726)	234 (217-244)	259 (234-287)
Conductivity (μmhos/cm at 25 C)	1097 (995-1240)	1067 (1010-1415)	358 (345-365)	467 (445-495)
Free CO_2 (mg/ℓ)	4.4 (2.4-6.6)	4.1 (1.6-6.9)	15.2 (9.6-20.6)	9.1 (7.3-12.2)

[a] Well water dilution used in all acute and embryo-larval bioassays except for the Omega-9 embryo-larval bioassay with rainbow trout.

[b] Dechlorinated tap-water dilution only used in Omega-9 embryo-larval bioassay with rainbow trout.

Table 4.2-3. Summary of acute, flow-through bioassay results for rainbow trout (Salmo gairdneri), fathead minnows (Pimephales promelas) and Daphnia pulex exposed to process waters and constituents in 1978.[a]

Process water or constituent	Species[b]	Number of organisms per concentration	Highest test concentration	TL_{50}	Observations
Process Waters					
Omega-9 oil shale retort water	Fathead minnows	20	0.8%	0.57%	Lethargic at 0.4%.
	Rainbow trout	20	0.8%	0.42%	Lethargic and loss of equilibrium at 0.4%.
	Rainbow trout[c]	--	--	0.51%[c]	Dechlorinated tap water for dilution.
	Daphnia pulex	10	0.8%	0.54%	Test duration was 48 hours.
Geokinetics -9 oil shale retort water	Fathead minnows	20	1.25%	0.88%	Hyperactive and sensitive to disturbance at 0.625%.
	Rainbow trout	20	1.25%	0.46%	Sensitive to disturbance at 0.313%.
	Daphnia pulex	10	1.25%	0.56%	Test duration was 48 hours.
Hanna-3 UCG condenser water	Fathead minnows	20	0.30%	0.11%	No signs of stress at sublethal concentrations.
	Rainbow trout	20	0.30%	0.10%	Hyperactive and gasping at the surface at 0.075%.
Constituents					
Omega-9 major inorganics[d]	Fathead minnows	20	1.6%	1.12%	Some loss of equilibrium and sensitivity at 0.8%.
	Rainbow trout	10	1.6%	0.56%	No signs of stress at sublethal concentrations.
Benzene	Fathead minnows	10	15.1 mg/ℓ	>15.1 mg/ℓ	Lethargic and sensitive to disturbance at 15.1 mg/ℓ.
	Rainbow trout	10	17.1 mg/ℓ	5.3 mg/ℓ	Rapid operculation and hyperactive at 3.8 mg/ℓ.
	Daphnia pulex	10	17.1 mg/ℓ	7.1 mg/ℓ	Test duration was 48 hours.

Table 4.2-3. (Continued)

Process water or constituent	Species[b]	Number of organisms per concentration	Highest test concentration	TL_{50}	Observations
Benzonitrile	Fathead minnows	20	167 mg/ℓ	64 mg/ℓ	Gasping at the surface, lethargic and loss of equilibrium at 50 mg/ℓ.
	Rainbow trout	20	154 mg/ℓ	32 mg/ℓ	Gasping at the surface and loss of equilibrium at 25 mg/ℓ.
	Daphnia pulex	10	154 mg/ℓ	<154 mg/ℓ	Test duration was 48 hours.
p-benzoquinone	Fathead minnows	20	1.0 mg/ℓ	0.04 mg/ℓ	Fish lethargic at 0.03 mg/ℓ.
	Rainbow trout	20	1.0 mg/ℓ	0.12 mg/ℓ	Fish exposed to 0.06 mg/ℓ were lethargic.
Hydroquinone	Fathead minnows	10	1.0 mg/ℓ	0.02 mg/ℓ	No sign of stress at sublethal concentrations.
	Rainbow trout	10	1.0 mg/ℓ	0.04 mg/ℓ	No sign of stress at sublethal concentrations.
	Daphnia pulex	10	1.0 mg/ℓ	0.77 mg/ℓ	Test duration was 48 hours.
Indene	Fathead minnows	20	25.9 mg/ℓ	18.2 mg/ℓ	At 10.2 mg/ℓ fish appeared lethargic.
	Rainbow trout	20	27.0 mg/ℓ	8.15 mg/ℓ	At 5.0 mg/ℓ fish appeared lethargic.
	Daphnia pulex	10	27.0 mg/ℓ	>27.0 mg/ℓ	Test duration was 48 hours.
Naphthalene[e]	Fathead minnows	20	12.7 mg/ℓ	8.95 mg/ℓ[e]	Test temp. 25°C; at 7.3 and 3.2 mg/ℓ loss of equilibrium and lethargic.
Phenol[f]	Fathead minnows	20	152 mg/ℓ	24.9 mg/ℓ[f]	Test temperature was 25°C.
	Daphnia pulex	10	109 mg/ℓ	>109 mg/ℓ	Test temperature was 25°C.

[a] All acute tests were conducted at 14°C for 96 hours and well water dilution, unless otherwise specified.

[b] Rainbow trout mean weight 15.6g and mean length 105.4mm; fathead minnow mean weight 0.93g and mean length 41.2mm.

[c] Omega-9 TL_{50} dilution of 0.51% was obtained using dechlorinated tap water as dilution water in a separate test; a value of 0.38-0.39% was reported earlier (1977 Annual Report) for the TL_{50} dilution in this test but the TL_{50} dilution was recalculated at 0.51% after all tests with low dissolved oxygen levels were discounted.

Table 4.2-3. (Continued)

[d] See Table 4.2-1 for concentrations of major inorganic constituents in test water.

[e] In an earlier test (1977 Annual Report) with naphthalene the fathead minnow TL_{50} value at 14°C was 4.9 mg/ℓ.

[f] In an earlier test (1977 Annual Report) with phenol the fathead minnow TL_{50} value at 14°C was 67.5 mg/ℓ.

Table 4.2-4. Summary of acute, static bioassay results for fathead minnows (Pimephales promelas) exposed to process waters and constituents.

Process water or constituent	Test duration	Exposure concentrations	Number of organisms per concentration	TL_{50}	Observations
Process waters					
Hanna-4A UCG condenser water	48 hr	0.1, 0.075, 0.05 and 0.025% in well water and well water control.	5	0.35%	Initially stressed at 0.025%.
Geokinetics-9 oil shale retort water	48 hr	0.5, 0.2, 0.1 and 0.5% in well water and well water control.	5	0.32%	Stressed at 0.1% and showed no signs of stress at 0.05%.
RS-12 pre-retort ground water	48 hr	10, 9, 8, 7 and 6% in well water and well water control.	5	7.6%	No mortality at 7 and 6%.
Constituents					
Aniline	48 hr	500, 300, 200, 100 and 50 mg/ℓ in well water and well water control.	5	142 mg/ℓ	No mortality in 100 and 50 mg/ℓ.
Benzonitrile	48 hr	250, 100, 10 mg/ℓ in well water and well water control.	3	163 mg/ℓ	No sign of stress at sublethal concentrations.
p-benzoquinone	48 hr	1.0, 0.5, 0.1, 0.05 and 0.01 mg/ℓ in well water and well water control.	3	0.02 mg/ℓ	No signs of stress at 0.01 mg/ℓ.
Indene	48 hr	25, 10 and 1.0 mg/ℓ in well water and well water control.	3	<25 mg/ℓ	

Table 4.2-4. (Continued)

Process water or constituent	Test duration	Exposure concentrations	Number of organisms per concentration	TL_{50}	Observations
Pyridine	72 hr	1000, 500 and 100 mg/ℓ in well water and well water control.	3	225 mg/ℓ	No sign of stress at 100 mg/ℓ.
Pyrrole	48 hr	500, 250 and 100 mg/ℓ in well water and well water control.	3	197 mg/ℓ	No sign of stress at 100 mg/ℓ.
Thiophene	48 hr	100 and 50 mg/ℓ in well water and well water control.	3	∿71 mg/ℓ	No sign of stress at 50 mg/ℓ.

The results of embryo-larval bioassays with Omega-9 oil shale retort water, Hanna-3 UCG condenser water, phenol and naphthalene are shown in Tables 4.2-5 through 4.2-8. The lowest dilution or concentration having any significant effect on egg hatchability, fry survival or fry growth was 0.16% for Omega-9 water, 0.01% for Hanna-3 water, 0.85 mg/ℓ for naphthalene, and 2.5 mg/ℓ for phenol.

In biochemical studies, no significant differences were seen between the activities of alkaline phosphatase, glutamine-pyruvate transaminase or glutamate dehydrogenase in the livers of rainbow trout exposed for 96 hours to 0.31% Omega-9 water and those in controls. Of the 22 metals assayed in the livers of rainbow trout exposed to 0.3% and 0% Omega-9 water for 96 hours, only iron showed a significant difference between exposed and control animals (Table 4.9-9). As shown in Table 4.9-10 effects on rainbow trout blood were observed at Omega-9 dilutions of 0.1% for packed cell volume, 0.31% for hemoglobin, 0.15% for plasma proteins, 0.30% for plasma ammonia, and 0.1% for plasma alkaline phosphatase activity. Exposure of rainbow trout for 96 hours to a 0.4% dilution of an artificial mixture of inorganics at levels found in Omega-9 water decreased packed cell volume about 10%, increased plasma ammonia about 3-fold, and significantly decreased plasma alkaline phosphatase activity (Table 4.2-11). The 96-hr TL_{50} dilution of this mixture in the rainbow trout test was 0.56% (Table 4.2-3).

In gill histopathology studies, the epithelial tissue of the secondary lamellae was separated from the pillar cell complex in rainbow trout exposed to the highest concentration of Hanna-3 condenser water. The lower concentrations also showed the same response but at each lower concentration the lesions were less pronounced. However, even at the lowest exposure (0.005% UCG condenser water) there was some epithelial separation, while the control filaments showed none. Electron microscopy observations indicate that the severe vacuolization seen by light microscopy in the exposed gill filaments is due primarily to a separation of the outer epithelial layer from the inner epithelial layer. Electron microscopy studies have also revealed "epithelial buds" on the surface of secondary lamellae of fish exposed to the highest concentration (0.075% condenser water). These "buds" appear to be the beginning of hyperplasic lesions, or epithelial cell sloughing.

The toxicant avoidance test system was set up and tested this year, and is now working well. It appears, in our first preliminary evaluation, that rainbow trout avoid a phenol concentration about one-third the 96-hr TL_{50} concentration.

Table 4.2-5. Summary of hatchability, survival and growth of rainbow trout (Salmo gairdneri) eggs and fry exposed to Omega-9 oil shale retort water and dechlorinated tap water control.[a]

Concentration[b] (% Omega-9 water)	Hatchability (%)	Survival at 69 days (%)	Mean length at 69 days (mm)	Mean weight at 69 days (g)
0	92	90	34.3	0.36
0.04	98	98	35.2	0.41
0.08	96	94	33.5	0.36
0.16	90	90	29.3[c]	0.24
0.26	94	86	24.5[c]	0.14
0.54	0	--	--	--

[a] Test temperature 15°C; water quality during test summarized in Table 4.2-2.

[b] Calculated on basis of diluter adjustment.

[c] Significantly different ($\alpha = 0.05$) from dechlorinated tap water control.

Table 4.2-6. Summary of hatchability, survival and growth of fathead minnow (Pimephales promelas) eggs and fry exposed to Hanna-3 UCG condenser water and well water control.[a]

Concentration[b] (% Hanna-3 water)	Hatchability (%)	Survival at 30 days (%)	Mean length at 30 days (mm)	Mean weight at 30 days (g)
0 (control)	83.5	48	19.3	0.12
0.0012	81.2	59	26.6	0.23
0.0025	76.4	51	25.4	0.22
0.005	79.3	47	25.0	0.21
0.01	67.7	8[c,d]	26.4	0.24
0.02	73.4	33[d]	24.5[d]	0.18
0.04	62.8[c]	10[c,d]	22.4[d]	0.13
0.08	19.0[c]	0	--	--

[a]Test temperature 25°C; water quality during test summarized in Table 4.2-2.

[b]Calculated on basis of diluter adjustments.

[c]Significantly different ($\alpha = 0.05$) from well water control.

[d]Significantly different ($\alpha = 0.05$) from fry reared in 0.0012% condenser water.

Table 4.2-7. Summary of hatchability, survival and growth of fathead minnow (Pimephales promelas) eggs and fry exposed to naphthalene and and well water control.[a]

Concentration[b] (mg naphthalene/ℓ)	Hatchability (%)	Survival at 30 days (%)	Mean length at 30 days (mm)	Mean weight at 30 days (g)
0 (control)	90.4	42	25.8	0.14
0.13	84.4	49	24.6	0.12
0.21	89.1	44	25.6	0.15
0.45	86.4	41	25.7	0.14
0.85	77.3[c]	51	23.5[c]	0.11[c]
1.84	88.6	52	22.8[c]	0.10[c]
4.38	65.8[c]	0	--	--
8.51	36.6[c]	0	--	--

[a]Test temperature 25°C; water quality during test summarized in Table 4.2-2.

[b]Concentration determined by weekly HPLC analysis.

[c]Significantly different ($\alpha = 0.05$) from well water control.

Table 4.2-8. Summary of hatchability, survival and growth of fathead minnow (Pimephales promelas) eggs and fry exposed to phenol and well water control.[a]

Concentration[b] (mg phenol/ℓ)	Hatchability (%)	Survival at 30 days (%)	Mean length at 30 days (mm)	Mean weight at 30 days (g)
0 (control)	81.8	52	22.9	0.27
0.23	82.7	31	22.8	0.18
0.75	81.4	44	22.8	0.25
2.5	78.2	50	21.1	0.19[c]
6.1	77.2	48	22.1	0.15[c]
14.5	72.3	26	20.3[c]	0.20[c]
33.2	77.8	0	--	--
68.5	45.2[c]	0	--	--

[a]Test temperature 25°C; water quality during test summarized in Table 4.2-2.

[b]Concentration determined by weekly HPLC analysis.

[c]Significantly different ($\alpha = 0.05$) from well water control.

Table 4.9-9. Element levels in livers of rainbow trout exposed to Omega-9 oil shale retort water; µg/g liver ± standard error of mean.

Metal	Omega-9 dilution 0.3%	0
Iron	44.6 ± 6.5[a]	67.1 ± 4.9
Potassium	3520 ± 110	3760 ± 100
Sodium	858 ± 39	922 ± 26
Calcium	49.8 ± 2.5	52.9 ± 3.6
Phosphorus	3.63 ± 0.10	3.70 ± 0.10
Copper	50.3 ± 3.4	50.8 ± 8.0
Magnesium	196 ± 5	196 ± 6
Zinc	26.8 ± 0.7	25.3 ± 0.7

[a]Significantly different ($\alpha = 0.05$) from control.

Table 4.2-10. Effects of 96-hr exposure to Omega-9 oil shale retort water on rainbow trout (Salmo gairdneri) blood parameters; mean ± SEM(N).

Concentration[a] (% Omega-9 water)	Packed cell volume (%)	Hemoglobin (g/100 ml)	Plasma protein (g/100 ml)	Plasma alk. phosphatase (μmoles/min/ℓ)	Plasma ammonia (μg/ml)
0 (control)	50.9 ± 1.1 (42)	10.0 ± 0.3 (10)	3.9 ± 1.2 (12)	124 ± 6 (63)	6.2 ± 1.1 (6)
0.08	50.8 ± 1.6 (10)	9.7 ± 0.3 (10)	3.4 ± 0.3 (4)	--	--
0.10	45.1 ± 1.7[b] (16)	--	--	106 ± 6[b] (40)	--
0.15	42.8 ± 1.4[b] (18)	--	3.2 ± 0.1[b] (8)	98 ± 8[b] (17)	9.5 ± 1.4 (8)
0.16	46.4 ± 2.1 (10)	9.0 ± 0.3 (10)	--	--	--
0.20	--	--	--	76 ± 6[b] (14)	--
0.30	39.8 ± 1.4[b] (19)	--	3.1 ± 0.1[b] (15)	78 ± 8[b] (15)	16.9 ± 2.5[b] (10)
0.31	41.8 ± 1.9[b] (10)	8.0 ± 0.5[b] (10)	--	--	--

[a]Calculated on basis of diluter adjustment.

[b]Significantly different ($\alpha = 0.05$) from control.

Table 4.2-11. Effect of 96-hr exposure to a solution of the major inorganic constituents in Omega-9 water on rainbow trout (Salmo gairdneri) blood parameters; mean ± SEM(N).

Parameter	Inorganic mixture dilution	
	0.4%	0%
Packed cell volume (%)	42.5 ± 1.6[a] (17)	47.5 ± 1.7 (19)
Plasma protein concentration (mg/100 ml)	3.38 ± 0.12 (20)	3.60 ± 0.08 (16)
Plasma alkaline phosphatase activity (μmoles/min/ℓ)	101 ± 8[a] (25)	132 ± 13 (20)
Plasma ammonia concentration (μg/ml)	13.1 ± 0.5[a] (14)	4.34 ± 0.16 (10)

[a]Significantly different ($\alpha = 0.05$) from control.

Interpretation

Oil Shale Retort Water

Acute toxicities of Omega-9 and Geokinetics-9 oil shale retort waters were remarkably similar. The TL_{50} dilution of Omega-9 in situ oil shale retort water ranged from 0.42 to 0.57% for the three aquatic species tested while the TL_{50} dilution of Geokinetics-9 water was 0.46 to 0.88% for the same species and test conditions (Table 4.2-3). Inspection of the chemical characteristics of Omega-9 retort water in Table 4.2-12 (similar data are not yet available for Geokinetics-9 retort water) suggests that inorganic constituents may contribute substantially to the acute toxicity of this oil shale retort water. At a dilution of 0.5% in our bioassays (in the range of our reported 96-hr TL_{50} values) the total organic carbon would have been only about 5 mg/ℓ. But the total ammonia concentration at a 0.5% dilution would have been about 18-19 mg/ℓ (as NH_3). At our test pH (about 8.0) and temperature (14°C) the percentage of total ammonia present in the toxic, un-ionized ammonia form would have been about 2.5% (Emerson et al. 1975), or 0.45 - 0.5 mg/ℓ un-ionized ammonia. Ball (1967) reported a 48-hr TL_{50} of 0.41 mg/ℓ un-ionized ammonia for rainbow trout, and Lloyd and Orr (1969) reported a similar 60-hr TL_{50} of 0.39 mg/ℓ un-ionized ammonia for the same species. Thus, the un-ionized ammonia concentrations present at Omega-9 TL_{50} dilutions were probably high enough to account for much, or even most, of the observed Omega-9 retort water toxicity.

The importance of inorganics in accounting for the acute toxicity of Omega-9 water was tested further using an artificial mixture of the inorganic constituents in the Omega-9 water (Table 4.2-1). The 96-hr TL_{50} dilution of this mixture was 0.56% for rainbow trout and 1.12% for fathead minnows (Table 4.2-3). These values are close to the Omega-9 96-hr TL_{50} dilutions of 0.42% for rainbow trout and 0.57% for fathead minnows tested under the same conditions (Table 4.2-3), indicating that the inorganics are indeed important constituents contributing to the acute toxicity of the Omega-9 retort water. Ammonia, with some possible contribution from high-concentration sulfur compounds (Table 4.2-12), undoubtedly contributes substantially to the acute toxicity of the inorganic fraction of Omega-9 water as well as the whole Omega-9 retort water.

In a 69-day embryo-larval bioassay with rainbow trout, the lowest concentration of Omega-9 water showing a significant effect was the 0.16% dilution, which reduced fry length to 85% of controls as shown in Table 4.2-13. This summary table also shows significantly reduced values for packed cell volume and plasma alkaline phosphatase activity in rainbow trout after exposure to 0.10% for 96 hours. This 0.10% dilution is the lowest Omega-9 concentration showing a significant effect on any parameter measured. The highest dilution tested having no significant effect on any parameter in the embryo-larval test was 0.08% Omega-9 retort water, and this dilution is shown tentatively as a maximum acceptable toxicant concentration (MATC) of Omega-9 water in Section 4.5, Recommendations.

UCG Condenser Water

Hanna-3 UCG condenser water was more toxic than Omega-9 or Geokinetics-9 oil shale retort water in 96-hr bioassays with rainbow trout and fathead minnows.

Table 4.2-12. Water quality characteristics of Omega-9 in situ oil shale retort water.[a]

Water quality parameter	Concentration[b] (mg/ℓ)
Alkalinity (as $CaCO_3$)	16,200 ± 480
Biochemical Oxygen Demand (5 day)	740
Carbon, Bicarbonate (as HCO_3^-)	15,940
Carbon, Carbonate (as $CO_3^=$)	500
Carbon, Inorganic (as C)	3,340 ± 390
Carbon, Total Organic (as C)	1,003 ± 192
Chemical Oxygen Demand	8,100 ± 5,700
Conductivity (μmhos/cm)	20,400 ± 3,840
Cyanide (as CN^-)	0.42 - 2.9
Hardness, Total (as $CaCO_3$)	110
Nitrogen, Total Ammonia[c] (as NH_3)	3,795 ± 390
Nitrogen, Kjeldahl (as N)	3,420 ± 420
Nitrogen, Nitrate (as NO_3^-)	0.17
Nitrogen, Organic (as N)	148 - 630
Oil and Grease	580
pH (unit)	8.65 ± 0.26
Phenols	60 ± 30
Phosphorus, Orthophosphate (as $PO_4^=$)	0.08 - 24.6
Solids, Fixed	13,430 ± 415
Solids, Total	14,210 ± 120
Solids, Total Dissolved	14,210 ± 193
Sulfur, Sulfate (as $SO_4^=$)	1,990 ± 250
Sulfur, Sulfide (as S)	0
Sulfur, Sulfite (as S)	<20
Sulfur, Tetrathionate (as $S_4O_6^=$)	280
Sulfur, Thiosulfate (as $S_2O_3^=$)	2,740 ± 730
Sulfur, Thiocyanate (as SCN^-)	123 ± 18

[a] From Fox et al. (1978).

[b] Where more than one measurement was made the mean ± 1 standard deviation is reported; a range is reported if the coefficient of variation was greater than 100%. A single value is reported for single analyses as in Fox et al. (1978).

[c] This is the sum of NH_3 and NH_4^+ expressed as mg NH_3/ℓ.

Table 4.2-13. Summary of effects on rainbow trout (Salmo gairdneri) exposed to sublethal concentrations of Omega-9 oil shale retort water or artificial mixture of major inorganic constituents in Omega-9 water; percent of control.

Parameter[a]	Exposure dilution (%)						
	Omega-9 oil shale retort water						Major inorganics
	0.08	0.10	0.15-0.16	0.20	0.26	0.30	0.04
Fry length	98	--	85[b]	--	71[b]	--	--
Packed cell volume	100	89[b]	84[b]	--	--	78[b]	89[b]
Plasma hemoglobin	97	--	90	--	--	80[b]	--
Plasma proteins	88	--	81[b]	--	--	78[b]	94
Plasma ammonia	--	--	154	--	--	274[b]	302[b]
Plasma alkaline phosphatase	--	85[b]	79[b]	61[b]	--	63[b]	77[b]
Liver iron	--	--	--	--	--	66[b]	--

[a]All tests are after 96-hr exposure (Tables 4.2-9, 10 and 11) except for fry length which was determined after a 69-day exposure (Table 4.2-5).

[b]Significantly different ($\alpha = 0.05$) from appropriate control.

As shown in Table 4.2-3 the 96-hr TL_{50} dilutions for the Hanna-3 water were 0.10% for rainbow trout and 0.11% for fathead minnows, while the TL_{50} dilutions ranged from 0.42% Omega-9 water for rainbow trout to 0.88% Geokinetics -9 water for fathead minnows.

The chemical characteristics of Hanna-3 condenser water (Table 4.2-14) show high concentrations of total organic carbon and ammonia, suggesting that these two constituents may have been important contributors to the acute toxicity of the Hanna-3 water. At the 96-hr TL_{50} dilution of about 0.1% the total organic carbon concentration would have been roughly 9 mg/ℓ. In the Hanna-3 condenser water one-half or more of the total organic carbon consisted of phenolic compounds, with phenol itself present at 2,300 mg/ℓ. Thus, at the 0.1% TL_{50} dilution of Hanna-3 water, the phenol concentration would have been about 2.3 mg/ℓ and the total phenolic concentration would have been roughly 4.5 mg/ℓ, assuming that 50% of the total organic carbon consisted of phenolic compounds. In 96-hr bioassays with phenol under conditions identical to those used in the Hanna-3 bioassays (14°C well water dilution) we determined TL_{50} values of 9 and 68 mg/ℓ for rainbow trout and fathead minnows, respectively (1977 Annual Report). These TL_{50} values for phenol are roughly 2 to 14 times higher than calculated phenolic concentrations at the observed TL_{50} dilution of about 0.1% Hanna-3 water. Therefore, although phenolic compounds may have contributed to the acute toxicity of Hanna-3 UCG condenser water, other constituents were probably more important.

The total ammonia concentration in Hanna-3 condenser water was approximately 20,000 mg/ℓ as NH_3 based on a single rough determination (Table 4.2-14). Based on this value, the total ammonia concentration (as NH_3) at the 0.1% TL_{50} dilution of Hanna-3 water could have been about 20 mg/ℓ. At our test pH of 8.1 and temperature of 14°C, un-ionized ammonia (the toxic species of ammonia) would have been 3.1% of the total ammonia concentration (Emerson et al. 1975) or about 0.6 mg/ℓ. As discussed earlier regarding Omega-9 oil shale retort water, the reported TL_{50} concentration of un-ionized ammonia is about 0.4 mg/ℓ for rainbow trout (Ball 1967, Lloyd and Orr 1969). Our roughly calculated concentration of un-ionized ammonia, 0.6 mg/ℓ, at the 0.1% TL_{50} dilution of Hanna-3 water was one and one-half times this reported TL_{50} value for un-ionized ammonia. Thus, based on the very approximate ammonia determination and calculated dilutions of this ammonia in our Hanna-3 96-hr bioassay, ammonia probably contributed importantly to the observed acute toxicity of Hanna-3 UCG condenser water.

Results from the Hanna-3 UCG condenser water embryo-larval bioassays with fathead minnows (Table 4.2-6) show that the lowest Hanna-3 concentration having a significant effect was the 0.01% dilution, where fry survival was reduced to 8% as compared to 48% survival in control well water. Significant effects were also seen at the 0.04% dilution for egg hatchability, and at the 0.02% dilution for fry growth. The significant reduction in fry length at 0.02% Hanna-3 water is based on a comparison with the lowest exposure dilution (0.0012%; Table 4.2-6) rather than the well water controls. This comparison was used because the fry reared in well water controls did not grow as well as the fry reared in low concentrations of condenser water. This reduced growth probably resulted from limited food availability in the control tanks; the test tanks supplied with even the lowest concentrations of condenser water contained substantial growths of microorganisms on which larval fathead minnows can feed, while such microorganism growth was not found

Table 4.2-14. Water quality characteristics of Hanna-3 UCG condenser water.

Water quality parameter	Concentration or value
Alkalinity (mg/ℓ as $CaCO_3$)	11,200
Carbon, Total Organic (mg/ℓ as C)	8,900
Cyanide (mg/ℓ)	0.05
Hardness (mg/ℓ as $CaCO_3$)	22
Nitrogen, Total Ammonia (mg/ℓ as NH_3)	20,000[a]
pH (units)	9.0
Phenol (mg/ℓ)	2,300

[a] This is the sum of NH_3 and NH_4^+ expressed as mg NH_3/ℓ. This value represents a single analysis which can no longer be verified because Hanna-3 water is no longer available. We have reason to suspect that this result is erroneously high because of interference by volatile amines in the electrode analysis.

in the control tanks. Although the fry reared in all test tanks were fed brine shrimp and powdered food daily, we apparently did not feed enough to control fry during this bioassay to negate the effect of varied food availability.

At the 0.01% dilution, the lowest concentration of Hanna-3 condenser water having a significant effect on any parameter in the fathead minnow embryo-larval biossay, the calculated concentrations of important constituents were 0.23 mg/ℓ for phenol, 0.45 mg/ℓ for total phenolics and 0.13 mg/ℓ for un-ionized ammonia (test temperature 25°C and pH 8.1). In our fathead minnow embryo-larval bioassay with phenol, the lowest concentration having a significant effect on any parameter was 2.5 mg/ℓ where mean weight of fry was reduced significantly compared to controls (Table 4.2-8). Concentrations of phenol as high as 0.75 mg/ℓ apparently had no effect on fathead minnow eggs and fry, suggesting that the phenol or, even, the total phenolic concentrations in the Hanna-3 embryo-larval test were not high enough to account for the level of Hanna-3 toxicity observed.

Other investigators have reported significant effects of low concentrations of un-ionized ammonia on growth of rainbow trout in long-term exposures. Burkhalter and Kaya (1977) found a significant reduction in growth of rainbow trout fry at un-ionized ammonia concentrations as low as 0.06 mg/ℓ and Smith and Piper (1975) reported reduced growth in rainbow trout exposed for six months to 0.033 mg/ℓ un-ionized ammonia. If the concentration of un-ionized ammonia in our 0.01% Hanna-3 dilution was indeed as high as the calculated value of 0.13 mg/ℓ, this ammonia level might account for much of the Hanna-3 condenser water toxicity in the fathead minnow embryo-larval bioassay.

Our preliminary evaluation of gill histopathology in rainbow trout exposed for 96 hours to Hanna-3 water also supports, if tentatively, an argument that ammonia is a major toxicant in Hanna-3 water. Dilutions as low as 0.005% Hanna-3 water caused epithelial lifting in the gill. Since 0.005% was the lowest dilution tested in this series of experiments, threshold-effect and no-effect dilutions were not determined for gill histopathology lesions. However, the calculated concentration of un-ionized ammonia at this dilution of Hanna-3 water is 0.065 mg/ℓ. And Smith and Piper (1975) reported rainbow trout gill lesions, similar to those we observed in 0.005% Hanna-3 water, at un-ionized ammonia concentrations down to 0.025 mg/ℓ, less than half the calculated un-ionized ammonia level in 0.005% Hanna-3 water.

There are, of course, certain problems with this evaluation of ammonia as a principal toxicant in Hanna-3 UCG condenser water. The single analysis of 20,000 mg/ℓ (as NH_3) total ammonia for Hanna-3 water was probably high due to interferences from volatile amines in the sample (see Table 4.2-14); calculated dilutions of ammonia no doubt tend to be erroneously high because of volatilization of ammonia in an intermittent-flow diluter such as those used in our toxicity tests; and synergistic or antagonistic toxicity among all of the constituents in UCG condenser water could be extremely important and we are unable to account for these effects. Nevertheless, based on all of the evidence gathered thus far, we suspect that ammonia, along with some possible contribution by phenolic compounds, accounts for the levels of Hanna-3 condenser water toxicity we have observed in acute and limited-chronic bioassays.

In the embryo-larval bioassay with fathead minnows, the highest concentration of Hanna-3 water showing no effect was the 0.005% dilution. This supports a

tentative maximum acceptable toxicant concentration (MATC) recommendation of 0.005% Hanna-3 water. However, the gill histopathology observed in rainbow trout exposed to 0.005% Hanna-3 water and the possibility of un-ionized ammonia concentrations being present at toxic levels in a 0.005% dilution, especially at temperatures at or above 25°C and pH at or above 8.0, both argue for a tentative MATC below 0.005%. Therefore, at least until we are able to analyze and test other UCG condenser waters, we recommend an MATC dilution of 0.003% for Hanna-3-like UCG condenser water (see Recommendations, Section 4.5).

Single Constituents

Naphthalene and phenol are both important constituents of coal gasification condenser water. Pellizzari (1978) quantified 1.8 mg/ℓ naphthalene (considering the volatile fraction only) in Hanna UCG condenser water, and we identified 2300 mg/ℓ of phenol in Hanna-3 condenser water. For this reason we selected these two compounds for embryo-larval bioassays with fathead minnows. As discussed in the preceding section and shown in Table 4.2-8, phenol affected fathead minnow growth at concentrations as low as 2.5 mg/ℓ. Primarily on the basis of this test we have recommended an MATC value of 0.75 mg/ℓ for phenol (see Section 4.5, Recommendations). Even though naphthalene is present in much lower concentrations than phenol in coal gasification condenser water, we still found reduced growth of fathead minnow fry at 0.85 mg/ℓ (Table 4.2-7) which is lower than the concentration found in coal gasification condenser water by Pellizzari. The 96-hour TL_{50} of naphthalene-exposed fathead minnows is 4.9 mg/ℓ at 14°C and 8.95 mg/ℓ at 25°C, so we would not expect the acute toxicity of naphthalene to be nearly as important to fish as long-term effects. On the basis of threshold-level effects of naphthalene at 0.85 mg/ℓ and a no-effect level of 0.45 mg/ℓ in the embryo-larval tests, we are recommending an MATC value for naphthalene at 0.45 mg/ℓ (see Section 4.5, Recommendations).

Since our flow-through 96-hour bioassays showed p-benzoquinone to be one of the most toxic constituents of coal gasification condenser water (Table 4.2-3), we chose that compound for an embryo-larval test with fathead minnows. But, when we began dosing the toxicant at the rate of 0.1 mg/ℓ (the 96-hour TL_{50} for fathead minnows was 0.022 mg/ℓ) the amount of p-benzoquinone that we actually measured during the first week of the test in our highest concentration tanks was only 0.032 mg/ℓ, and we could not detect any p-benzoquinone in the lowest five dosed tanks. This degradation seemed to be time-dependent, because during the second week of the test we were not able to detect any p-benzoquinone in any test tanks. Since our dosing system was working properly, we concluded that a bacterial population had been established in the tanks which degraded the dosed p-benzoquinone. In conjunction with these findings we observed that hatchability was not impaired in any of the test dilutions, and after three weeks of fry exposure there did not appear to be any differences in survival or growth between control fry and those in any dosed tank, so we terminated the experiment. The results from this short experiment are important because they suggest that although p-benzoquinone is a highly toxic constituent of coal gasification condenser water it is not likely to cause important environmental problems unless it is released as a large spill because it is apparently easily degraded.

4.2.2 Work in Progress

We are currently completing an embryo-larval bioassay to determine the effects of phenol on rainbow trout, and we are completing all work on gill histopathology begun during 1978. Also, we are preparing several manuscripts for publication on the work completed thus far (see Section 5.5).

4.2.3 Work to be Performed Next Year

During the 1979 calendar year we will alter the emphasis of our research somewhat to screen at least ten advanced fossil-fuel process waters for acute toxicity and three for limited-chronic toxicity as set forth in the Year-3 Task Agreement.

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

4.3.1 Work Completed This Year

A microbial degradation test system for evaluating environmental persistence of organic compounds was developed this year. Using phenol as a model compound, we conducted an orthogonal, fractional-factorial analysis of several parameters to define the optimal experimental strategy. Evolved from this study is a shake-flask system which we are employing to evaluate the biodegradability of process waters from advanced fossil fuel technologies and individual compounds known to occur in these waters.

Methods Synopsis

For the orthogonal, fractional-factorial investigation of microbial degradation systems, each experimental unit consisted of a flask containing phenol exposed to varying environmental parameters. The interactions of six treatments were tested at two to three levels each; testing for interactions at each level was inherent in the design. Parameters and levels evaluated were:

1) Type of basal salts medium (BSM)

   a) A medium for detecting phenol-degrading bacteria, pH 8 (Ralston and Vela 1974)

   b) A medium for bacteria oxidizing liquid hydrocarbons, pH 7-7.2 (Rodina 1972)

   c) Voroshilova and Dianova medium, pH 7 (Rodina 1972)

2) Amount of hydrocarbon substrate (phenol)

   a) 100 mg/ℓ

   b) 200 mg/ℓ

   c) 400 mg/ℓ

3) Microbial source

   a) Oil refinery settling pond sludge

   b) Prefiltered settling pond sludge

4) Amount of microbial inoculum

   a) 1 ml

   b) 5 ml

   c) 10 ml

5) Initial pH

   a) 1 pH unit above BSM recipe

   b) pH unit of recipe

   c) 1 pH unit below BSM recipe

6) Aeration

   a) Screw-cap flask

   b) Cotton stoppered flask

Basal salts media were added to each experimental 250-ml Erlenmeyer flask and autoclaved. Following cooling, the addition of sterile phenol solution and a microbial inoculum brought the volume in each flask to 200 ml. The flasks were incubated at 30°C under constant light on a rotary shaker at 100 rpm. Samples were collected from the flasks using a sterile syringe, preserved with $H_3PO_4/CuSO_4$ solution and analyzed within 24 hr by high performance liquid chromatography (HPLC). Depending on previous analyses during each experimental run, samples were collected every 8 hr the first day and every 4-6 hr thereafter, each run lasted 96-100 hr.

Work-up and statistical analyses of the data from the 81 experimental units proceeded through the following steps:

1) Peak heights from the HPLC analyses of each flask over time were translated both to phenol concentrations (mg/ℓ) and to percent concentration of the control flasks.

2) The data were plotted as concentration vs. elapsed time and used to build a computerized data file.

3) Best-fit polynomial equations were determined for each curve via a BMD P5R package program.

4) From the equations, we calculated slopes of the lines during both the lag period and the maximum degradation period, the time to 90% phenol degradation and the percent of phenol degradation at 24, 48, 72 and 96 or 100 hr.

5) Significant differences and interactions between flasks, parameters and levels were determined through an orthogonal analysis program authored by Donald Anderson of the U.W. Statistics Department.

Based on the statistical analysis in Step 5, the optimum phenol degradation system was defined for the parameters in Step 4. We then applied this system to kinetic analyses of naphthalene and benzene metabolism and to the degradation of Omega-9 and Geokinetics-9 oil shale process waters.

For the single compound tests 195 ml of Ralston and Vela BSM at pH 8, 5 ml of bacterial inoculum and 100 µl of a methanol solution containing the compound were added to 250-ml teflon-capped flasks. Both unfiltered oil refinery settling pond sludge (Sinclair, Wyoming) and water from a natural pond (LaBonte Lake, Laramie) were used as inocula to evaluate potential environmental degradability over a wide range of conditions. Triplicate flasks were run with a single control, sampled at 0, 48, 72, 96, 144, 192 and 240 hours and analyzed by HPLC.

Six treatments for each process water degradation series were prepared with 175 ml of Ralston and Vela BSM. Additions of Sinclair refinery settling pond bacterial inoculum, distilled water and process water were varied as indicated in Table 4.3-1. Treatments 1-5 were prepared in sterile, 250-ml screw-cap flasks and agitated at 100 rpm on a rotary shaker in constant 4300-lux white fluorescent light at 28 ± 2°C. As a control, Treatment 6 was prepared in a sterile, brown screw-cap bottle and refrigerated at 4°C. Samples were removed from each flask at 10-day intervals, filtered through a sample clarification kit and collected in Teflon-capped vials. HPLC gradient analyses were conducted under the conditions listed in Table 4.3-2 and analyzed for disappearance and appearance of peaks with time.

Results

From the orthogonal, fractional-factorial analysis we concluded that: (1) Ralston and Vela medium and either 5 or 10 ml of inoculum most rapidly degraded a small concentration of substrate (100 ppm for phenol). (2) The optimum pH for the most rapidly degrading system was dependent upon amount of inoculum. If 5 ml inoculum was used, pH 8 was faster; if 10 ml was used, then pH 7 was more rapid. (3) Unfiltered inoculum yielded faster degradation at 10 ml, and no difference at 5 ml, compared to filtered inoculum. (4) Aeration had no effect on degradation. (5) Temperature, light, and shaking should be maintained as previously described. (6) Photodegradation did not occur in the control flasks and need not be considered a significant factor in phenol loss. We then explored the shake-flask system evolved from this analysis by performing three single-compound and two whole process water degradation tests.

Bacterial inocula from the refinery settling pond reduced benzene concentrations to less than 90% of the initial concentration by 192 hr, while naphthalene was completely consumed by 48 hr and phenol disappeared by 96 hr. The bacterial inocula from LaBonte Lake had no effect on either benzene or naphthalene by 240 hr, while phenol was degraded by 192 hr. In all of these experiments, compounds in the control flasks maintained initial concentrations.

The initial HPLC gradient chromatograms of Treatment 1 for Geokinetics-9 Omega-9 process waters are presented in Fig. 4.3-1 and 4.3-2. Presently we have completed the analyses through the 60-day samples for the Omega-9 treatments and the 20-day samples for the Geokinetics treatments. Since the degradation of Geokinetics-9 samplescannot be meaningfully interpreted yet, only the Omega-9 series will be discussed.

Table 4.3-1. Contents of the six treatment flasks for each long-term process water degradation study.

Treatment number	Process water volume (ml)	Sinclair inoculum volume (ml)	Sterile d·H_2O volume (ml)	R+V BSM volume (ml)	Total volume (ml)
1	20	5	0	175	200
2	4	5	16	175	200
3	2	5	18	175	200
4	20	0	5	175	200
5	0	5	20	175	200
6	20	0	5	175	200

Table 4.3-2. HPLC conditions for gradient analyses of long-term process water degradation samples.

Instrument: Waters Associates Model 204 HPLC

Column: 30 cm μ-C_{18}, reverse-phase

Detector: 254 nm UV; 0.1 AUFS

Injection sizes: 0.5 ml-2.0 ml

Gradient: 60 min, linear; 0-100% CH_3CN/H_2O

Flow rate: 2.0 ml/min

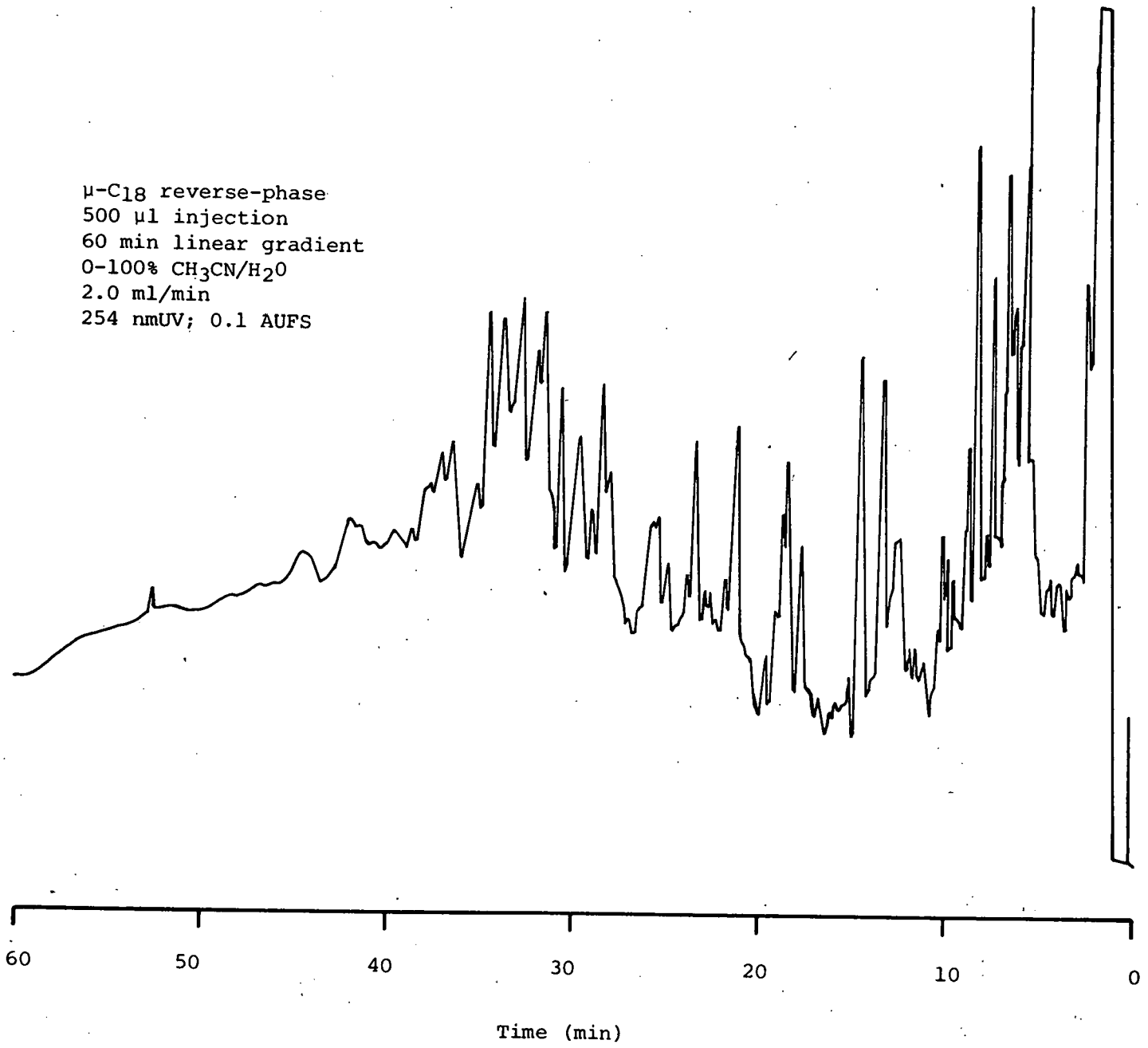

Figure 4.3-1. HPLC gradient chromatogram of initial Geokinetics-9 Treatment #1.

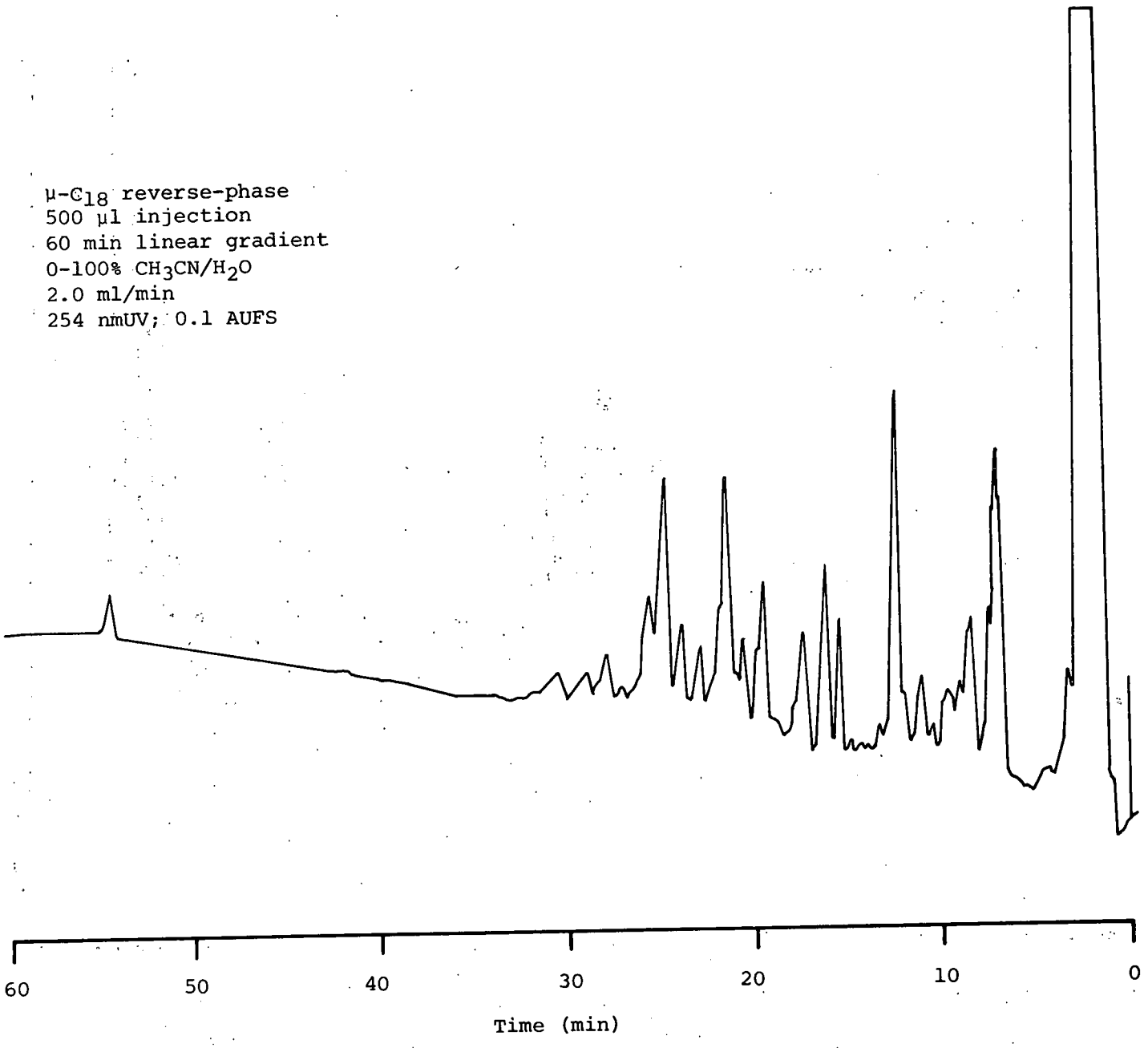

Figure 4.3 2. HPLC gradient chromatogram of initial Omega-9 Treatment #1.

Fig. 4.3-3 presents the gradient chromatogram of the 60-day Omega-9 Treatment #1 sample. A comparison with the initial gradient in Fig. 4.3-2 illustrates the typical trends of the bacterially mediated degradation of the process water components. Though we presently do not possess the electronic integration capability necessary to quantitatively evalute such complex chromatograms, several general observations can be stated:

1) All major peaks in the Omega-9 samples displayed retention times less than 32 minutes, indicating relative polarities equal to or greater than that of naphthalene.

2) With few exceptions, peaks present in the initial chromatograms decreased in height over time.

3) On the 20-day chromatograms of the bacterial Treatments 1-3, peaks became broad and poorly resolved, compounding the difficulty of interpreting process water degradation.

4) As the original peaks decreased in height and disappeared, new peaks often simultaneously appeared with shorter retention times in the more polar region of the chromatograms.

5) The control Treatments 4 and 6 displayed losses of early-eluting peaks and reductions in the more stable late-eluting peaks, though the changes were minor compared to those in the bacterial treatments.

Interpretation

The biodegradation system we have characterized is an integral part of a screening system to evaluate the environmental hazard of hydrocarbons produced during advanced fossil fuel processing technologies. Recalcitrant compounds, process water fractions or whole process waters, identified from the first screening, will be further examined with altered test systems (e.g., less concentrated substrate, different microbial source, etc.). Compounds which repeatedly prove not to be biodegradable will probably have to be treated by physical/chemical methods if concentrations in process water effluents prove to be toxic. Conversely, if a compound is shown to be biodegradable, and if its metabolites are not more toxic than the original compound, biological treatment should suffice. Kinetic studies of the compounds' biodegradation should help in the design of advanced control technologies for produced effluents.

Preliminary results using the compounds naphthalene and benzene indicate that the system will work for the analyses of compounds other than phenol with a promising degree of reproducibility. We have qualitatively concluded that the Omega-9 process water contained relatively polar organics degradable by bacteria from a refinery settling pond. As the original compounds disappeared from the gradient chromatograms, higher polarity metabolites appeared and subsequently degraded. Most process water degradation occurred between 10 and 20 days of incubation, after which little change was noted.

However, quantitative analysis of process water gradient chromatograms will require more sophisticated techniques than we presently employ. To this end we

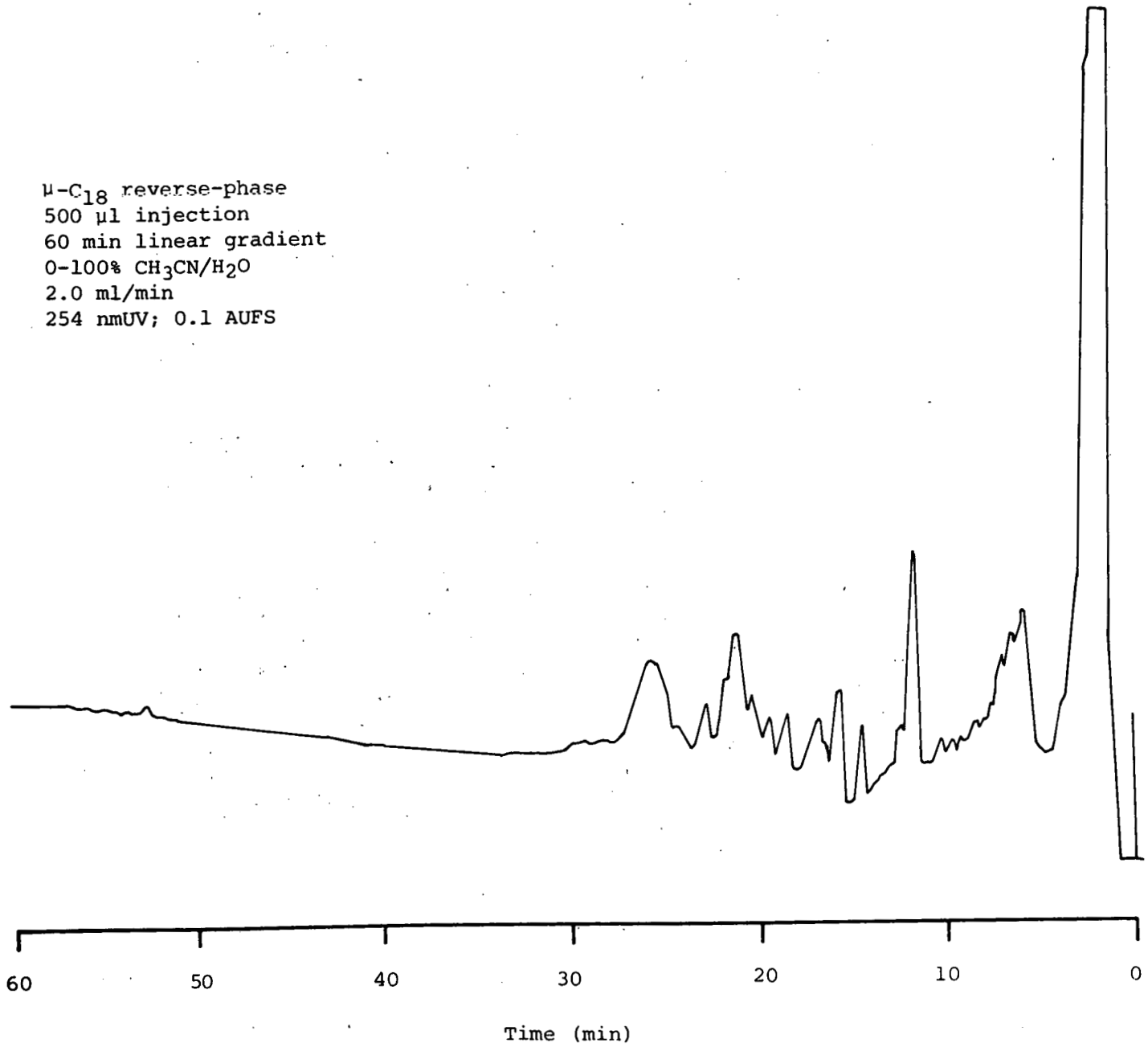

Figure 4.3-3. HPLC gradient chromatogram of 60-day Omega-9 Treatment #1.

plan to improve our HPLC detection system with electronic integration and interpret changes in the chromatograms with supplemental total organic carbon and gas chromatographic-mass spectrometric analyses.

4.3.2 Work in Progress

We are continuing to monitor the long-term degradation treatments of Omega-9 and Geokinetics-9 process waters until the end of December. As whole process waters and their fractions will be analyzed more extensively next year, we are preparing an environmental chamber and a larger capacity rotary shaker system that will provide more uniform conditions for our shake-flask experiments.

4.3.3 Work to be Performed Next Year

Long-term degradation experiments with Omega-9, Geokinetics -9 and other process waters as they become available will be initiated. Additional treatments with inocula from other sources, such as the Laramie River, will be added to the series so that potential environmental degradability of process waters can be evaluated over a wider range of conditions. We also hope to improve our quantitation of the degradation processes with electronic integration of the HPLC gradient chromatograms and total organic carbon analyses of the effluent waters. Single compound degradations will continue to be analyzed toward the goal of refining, as necessary, our degradation test protocol and evaluation criteria.

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

4.4.1 Work Completed This Year

Procedures for extraction of phenol from rainbow trout and fathead minnows were investigated this year. By exposing fish to ^{14}C-phenol, we were able to verify extractions for each method. A tissue homogenization/solvent extraction technique provided unacceptably low efficiency, but acceptable results were obtained by exhaustive steam distillation. We experienced problems injecting large volumes of n-octanol, our initial extract reservoir solvent choice, onto liquid chromatography columns and had to investigate other steam distillation solvents.

Methods Synopsis

Groups of rainbow trout and fathead minnows weighing 10-20g were exposed to sublethal concentrations of ^{14}C-phenol for up to 24 hours in static bioassays. As a preparation for both extraction methods, the fishes were sacrificed and cut into small pieces.

Equal weights of fish and Na_2SO_4 were homogenized for 5 minutes in the first step of the tissue homogenization/solvent extraction procedure. To this homogenate were added 25 ml of either chloroform ($CHCl_3$) or tetrahydrofuran (THF), then homogenization was continued an additional 10 minutes. Filtration of the extract liquid preceded injection into a high pressure liquid chromatograph (HPLC) for resolution and quantitation of phenol.

As an alternative procedure, the exhaustive steam distillation methods described for pesticides and PCB's by Veith and Kiwus (1977) was adapted for use with phenol. We attempted to modify the column design to improve extraction efficiency and tested toluene, n-butyl ether and n-octanol as potential extract reservoir solvents. Briefly, our procedure involved boiling either the fish or a known addition of phenol in 1% H_2SO_4 under reflux for up to 24 hours. The refluxed condensate passed through the extraction solvent before returning to the boiling flask, thus effecting the extraction of distilled phenol while leaving a majority of the non-volatile tissue components behind in the boiling flask. Phenol content of the extract reservoir solvent was quantified by reverse-phase HPLC.

Samples of the homogenized tissue or aliquots of the boiling flask potpourri were prepared for liquid scintillation counting (LSC) along with their corresponding extract liquids. Protosol (New England Nuclear) to digest tissue and appropriate volumes of PPO/POPOP/Toluene/X-100 LSC solution were added to the scintillation vials prior to ^{14}C assays on a Beckman LS-100C Scintillation System.

Results

A 9% extraction efficiency was determined by radiolabelled assays for the $CHCl_3/Na_2SO_4$ homogenization of rainbow trout exposed to 5.5 mg/ℓ phenol for 16 hours. With THF/Na_2SO_4 homogenization, only traces of phenol were extracted.

In contrast, for steam distillation with n-octanol as the extract reservoir solvent we calculated 80% extraction efficiencies by radiolabelled assays.

Rainbow trout were exposed to 10 mg/ℓ phenol for 16 hours in these tests. This relatively high efficiency compares well with the maximum of 88% extraction efficiency we determined for distillation of aqueous phenol samples. For various refluxing times with toluene and n-octanol, efficiencies of both the original and modified column designs are presented in Table 4.4-1.

Interference from extraneous tissue components distilling with the phenol is minimal in the chromatograms of n-octanol extracts. However, our attempts to quantify low phenol concentrations were hampered by an inability to inject large volumes of n-octanol onto either μ-C_{18} or μ-Porasil HPLC columns without appreciably spreading the peaks and decreasing resolution. We have substituted n-butyl ether as the extract reservoir solvent, but have not yet analyzed the extractions of radiolabelled phenol to compare with the performance of n-octanol.

Interpretation

Although McKee and Tarazi (1974) reported 67-75% homogenization extraction efficiencies from shrimp and oysters spiked with phenol, we have confirmed only a 9% efficiency from rainbow trout exposed to sublethal concentrations of ^{14}C-phenol. Based on his experience with chlorinated phenols, Dr. Donald Clark (personal communication) has suggested that phenol may bind with proteinaceous tissue and does not partition extensively into lipids. Thus, organic solvent homogenization cannot adequately remove this incorporated phenol from the tissue and low extraction efficiencies are obtained. We do not believe that extractions of spiked tissue provide a reliable efficiency confirmation for the bioaccumulation of phenolic compounds.

Exhaustive steam distillation, though more expensive and time consuming, appears to be the only acceptable technique for extracting phenol from fish. n-Octanol provided high efficiencies from both phenol-contaminated aqueous samples and rainbow trout exposed to ^{14}C-phenol. Since distillations longer than 12 hours did not improve extraction, we appear to be limited to 88% and 80% efficiencies, respectively, for the aqueous and fish samples.

For selection of the extract reservoir solvent, five properties were considered: (1) insolubility in water, (2) specific gravity less than 1.0, (3) high partition coefficient of phenol from water, (4) low UV absorbance and (5) compatibility with an HPLC column. n-Octanol satisfies all these criteria except the last when large volumes must be injected onto an HPLC to quantify low phenol concentrations. We suspect the high viscosity of this solvent inhibits mass transfer of phenol during its elution through the column and, combined with its chemical affinity for phenol, spreads the chromatographic peaks and decreases resolution. Although extraction efficiencies will decrease, we predict that n-butyl ether will be compatible with a normal-phase HPLC column and will be an acceptable extract reservoir solvent. We also predict that n-butyl ether, in combination with a non-polar solvent, will be a good extract reservoir solvent for samples contaminated with whole process waters.

Table 4.4-1. Steam distillation extractions of phenol-contaminated water.

Extraction solvent	Refluxing time (hr)	Extraction efficiency (%)	
		Original design	Modified design
Toluene	4	44	--
n-Octanol	4	80	--
n-Octanol	12	88	76 81
n-Octanol	24	88	85 76

4.4.2 Work in Progress

Radiolabelled phenol extraction efficiencies from fish are being determined by exhaustive steam distillation using n-butyl ether as the extract solvent.

4.4.3 Work to be Completed Next Year

Analysis of phenol extractions will continue. If n-butyl ether is an acceptable extraction solvent for phenolics, combinations with non-polar solvents (hexane, cyclohexane or iso-octane) will be evaluated to determine a solvent mixture suitable for exhaustive steam distillation of fish exposed to whole process waters and fractions. Extraction efficiencies will be calculated for fish exposed to uniformly tritiated process waters, if they are available, or by comparison with exhaustive steam distillations of process-water-contaminated aqueous samples.

4.5 Task 5: Recommendations (Marcus, Bergman, DeGraeve, J. Anderson)

With the completion this year of four embryo-larval, limited-chronic bioassays, we can estimate maximum acceptable toxicant concentrations (MATC) for Omega-9 oil shale retort water, Hanna-3 UCG condenser water, phenol and naphthalene. MATC values are specifically for the individual test species under the bioassay conditions employed. However, the values provide very good comparisons for relative toxicity levels of different toxicants. Mount and Stephan (1967) have proposed that MATC values can be used to calculate "application factors" which, in turn, can be used to estimate "safe concentrations" from acute bioassays with other species. An application factor is computed by dividing the species' MATC value for a toxicant by its TL_{50} value determined for the same toxicant (Committee on Water Quality Criteria 1973). Multiplying other species' TL_{50} values for the toxicant by the application factor determined for the first species will then provide estimates of safe concentrations for the other species. We have also extended the use of these application factors to estimate safe concentrations for species tested only in acute bioassays with 11 additional toxicants which appear to be chemically and biologically similar to the process waters or constituents we tested in embryo-larval bioassays.

The MATC values for process waters from advanced fossil fuel processing technologies indicate that UCG condenser water may be almost an order of magnitude more toxic than in situ oil shale retort water (Table 4.5-1). These MATC values provide a basis to project the potential hazards presented to natural aquatic systems in the event of accidental releases of untreated process waters or in the event that contaminated ground waters communicate with surface waters. With such events, it currently appears that the dilution factor necessary to protect natural aquatic communities may be much larger for UCG condenser water than for in situ oil shale retort water.

In embryo-larval bioassays with fathead minnows, MATC values of 0.75 and 0.45 mg/ℓ were determined for phenol and naphthalene, respectively (Table 4.5-1). Based on these values and also the TL_{50} values for phenol (24.9 mg/ℓ) and naphthalene (8.95 mg/ℓ), application factors for fathead minnows assayed at 25°C were determined to be 0.03 for phenol and 0.05 for naphthalene. According to the EPA Blue Book (Committee on Water Quality Criteria 1973:122),

> Where toxicants have a nonpersistent nature (a half life of less than 4 days) or noncumulative effects, an application factor of 0.1 of the 96-hour LC50 should not be exceeded at any time or place after mixing with the receiving waters. The 24-hour average of the concentration of these toxicants should not exceed 0.05 of the LC50 if aquatic life is to be protected. For toxic materials which are persistent or cumulative the concentration should not exceed 0.05 of the 96-hour LC50 at any time or place and the 24-hour average concentration should not exceed 0.01 of the 96-hour LC50 in order to protect aquatic life.

Therefore, based on the Blue Book recommendations and application factors we determined for phenol and naphthalene, we selected an application factor of 0.05 to estimate the safe concentrations of 11 other compounds for which we determined

Table 4.5-1. Summary of recommendations on maximum acceptable toxicant concentrations (MATC) and estimated safe concentrations (ESC) for process waters and single constituents tested in 1977-1978.

Process water or constituent	Acute toxicity bioassays: Most sensitive species	Acute toxicity bioassays: TL_{50}	Embryo-larval bioassay: species[a]	Embryo-larval bioassay: Most sensitive measure(s)	Embryo-larval bioassay: Threshold effects concentration	Embryo-larval bioassay: MATC	Recommendations: Application factor[b]	Recommendations: Estimated safe concentration	Comments
Process Waters									
Omega-9 oil shale retort water	RBT	0.42%	RBT	length	0.16%	0.08%	0.2	0.08%	
Geokinetics-9 oil shale retort water	RBT	0.46%					0.2	0.09%	Based on appl. fact. for Omega-9
Hanna-3 UCG condenser water	FHM[c]	0.11%	FHM	survival gill histo.	0.01% 0.005%	0.003%	0.03	0.003%	
Constituents									
Benzene	RBT	5.3 mg/ℓ					0.05	0.3 mg/ℓ	
Phenol	FHM[d]	24.9 mg/ℓ	FHM	weight	2.5 mg/ℓ	0.75 mg/ℓ	0.03	0.75 mg/ℓ	U.S.EPA (1976) recomm. 1 µg/ℓ Bergman et al. (1979) recomm. 0.5-1 mg/ℓ
Catechol	FHM	3.5 mg/ℓ					0.05	0.2 mg/ℓ	
Resorcinol	FHM	∿100 mg/ℓ					0.05	5.0 mg/ℓ	
Hydroquinone	FHM	0.02 mg/ℓ					0.05	0.001 mg/ℓ	
o-Cresol	RBT	8.4 mg/ℓ					0.05	0.4 mg/ℓ	
m-Cresol	RBT	8.9 mg/ℓ					0.05	0.4 mg/ℓ	

Table 4,5-1, (Continued)

Process water or constituent	Acute toxicity bioassays: Most sensitive species	Acute toxicity bioassays: TL_{50}	Embryo-larval bioassay: Most sensitive species[a]	Embryo-larval bioassay: Most sensitive measure(s)	Embryo-larval bioassay: Threshold effects concentration	Embryo-larval bioassay: MATC	Recommendations: Application factor[b]	Recommendations: Estimated safe concentration	Comments
p-Cresol	RBT	8.6 mg/ℓ					0.05	0.4 mg/ℓ	
Benzonitrile	RBT	32 mg/ℓ					0.05	1.2 mg/ℓ	
p-benzoquinone	FHM	0.04 mg/ℓ					0.05	0.002 mg/ℓ	
Naphthalene	FHM[e]	8.95 mg/ℓ	FHM	hatchability, length, weight	0.85 mg/ℓ	0.45 mg/ℓ	0.05	0.45 mg/ℓ	
Indene	RBT	8.15 mg/ℓ					0.05	0.4 mg/ℓ	

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

[b] Application factors for Omega-9, Hanna-3, phenol and naphthalene all computed from TL_{50} and MATC; all others 0.05 unless otherwise noted in comments (also see text for discussion).

[c] Rainbow trout were more sensitive with 96-hr TL_{50} at 0.10% condenser water, but fathead minnow value (0.11%) used for computation of MATC.

[d] Rainbow trout were more sensitive with 96-hr TL_{50} at 8.9 mg phenol/ℓ, but fathead minnow 96-hr TL_{50} value at 25°C (24.9 mg phenol/ℓ) used for computation of MATC.

[e] Rainbow trout were more sensitive with 96-hr TL_{50} at 2.2 mg naphthalene/ℓ, but fathead minnow 96-hr TL_{50} value at 25°C (8.95 mg naphthalene/ℓ) used for computation of MATC.

acute toxicities but not MATC values (Table 4.5-1). The estimated safe concentrations (ESC) provide preliminary target criteria on which to base the design of process water treatment systems for proposed fossil fuel processing technologies. The ESC value for a compound is based on that compound separately in solution; synergistic relationships with other toxicants are not considered.

Final recommendations for maximum permissible concentrations of compounds released in aqueous effluents from fossil-fuel technologies will be presented at the completion of Year-3 studies. These recommendations will be based on our research results as well as on a comprehensive survey of the literature.

5.0 ADDITIONAL ACTIVITIES

5.1 Visitors Received

Dr. James V. Ward, Department of Zoology and Entomology, Colorado State University, Ft. Collins, visited the group in April and discussed his work concerning the impacts of oil shale development on stream ecosystems.

Dr. John Giesey and Mr. John Bowling from Savannah River Ecology Laboratory, Aiken, South Carolina visited in May to discuss possible collaboration on an EPA - Athens funded project that they will be beginning in July. They will be evaluating fate and effects of a series of fossil energy related organics in model streams.

Dr. Frank Sanders, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee visited the group on 26 May to obtain information required for an analysis being compiled on the environmental impact of fossil fuel process waters.

Dr. Carl Gehrs, also from the Environmental Sciences Division at Oak Ridge National Lab, visited the group as well as Farrier and Kerr at LERC from 4-6 June. The purpose of his visit was to discuss EPA-DOE-HEW sponsored research priority conferences on environmental effects of advanced fossil fuel processing technologies and to discuss possible collaborative studies on degradation of organics found in fossil fuel process waters.

Dr. Charles A. S. Hall, Cornell University, Ithaca, NY, visited from 30 June to 4 July to discuss environmental analysis of advanced energy technologies.

Dr. Alan Maki, Procter and Gamble, Cincinnati, Ohio, visited the group in early July to discuss aquatic toxicology methodologies and the objectives of the American Society for Testing and Materials--Aquatic Toxicology Section.

Drs. John Giesey and James Alberts from Savannah River Ecology Laboratory, Aiken, South Carolina visited the group in early July. They discussed their work with the metal binding capacities of organic compounds and continued earlier discussions of Giesey's work with the fate of energy-related organic compounds in model stream ecosystems.

Mr. Daniel Woodward and Mr. Tom Jackson of the Columbia National Fishery Research Laboratory, U.S. Fish and Wildlife Service, visited the project on 7 August to review the project's goals and accomplishments.

Dr. James McKim of the EPA Environmental Research Laboratory, Duluth, Minnesota, visited the group on 18 August and discussed advances in bioassay techniques and reviewed the project goals and progress.

Dr. Ken Biesinger, EPA Environmental Research Laboratory, Duluth, Minnesota, and an EPA Project Officer for this project, visited in September to review problems and progress. A meeting was held with Dr. Biesinger, Dr. David Farrier (DOE-LETC) and all project personnel present to overview all project tasks.

Robert Ireland and David Rice from the Environmental Sciences Division, Lawrence Livermore Laboratory (LLL) visited on 13 and 14 December to discuss the possibility of our doing process water screening bioassays on LLL's Rio Blanco oil shale retort water. Ireland also met with Dr. David Farrier (TPO at LETC) regarding the same matter.

5.2 Response to Inquiries

Dr. Frank Sanders of the Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, contacted Bergman in February concerning the general scope and findings of our research. This information was required for an analysis being compiled on the environmental impact of fossil fuel processes.

Ms. Frances Connor, who is associated with the EPA Energy Assessment Project, Division of Natural and Physical Sciences, University of Colorado at Denver wrote us on 10 July and 1 August requesting information on our project. Copies of our quarterly and annual reports were sent.

Mr. Ben Parkhurst of the Environmental Sciences Division, Oak Ridge National Laboratory, inquired on 2 August about the equipment we are using for flow-through bioassays since they are considering setting up similar systems. Information was provided on our proportional diluter designs, delivery systems, pump brands, etc.

Mr. John Bowling of the Savannah River Ecology Laboratory called in early August concerning the status of our bibliography as they are interested in incorporating some of the material in the bibliography in their work with energy-related organic compounds. He was updated on the bibliography's progress.

Mr. Bruce Smith of the Bureau of Land Management in the Rock Springs, Wyoming office called to inquire about aquatic toxicity procedures that we use and asked for assistance in interpreting a possible aquatic contaminant problem in the Green River due to oil drilling. His questions were answered on the telephone.

Dr. Howard E. Johnson, Michigan State University, and colleagues called by conference telephone to ask for information on: (1) analytical procedures for phenolic compounds; (2) toxicities of phenolic compounds to fish; and (3) availability of published literature on fate and effects of certain phenolic compounds. All questions were related to an artificial stream study these investigators are discussing with EPA-Duluth. Most questions were answered by telephone and our phenolic analytical procedures and bibliography were sent to Dr. Johnson.

5.3 Meetings Attended

Waters Associates Liquid Chromatography School, Milford, Massachusetts, 9-12 January 1978.

-Meyer

Bibliographic Retrieval Services, Inc. training session, Denver, Colorado, 17-18 January 1978.

-Marcus

Colorado-Wyoming Chapter, American Fisheries Society Annual Meetings, Ft. Collins, Colorado, 1-2 March 1978.

-Bergman, DeGraeve, Fannin

Fish Culture and Disease Workshop, U.S. Fish and Wildlife Service, Spearfish, South Dakota, 13-24 March 1978.

-DeGraeve

EPA Workshop on Degradation of Organic Compounds in Aquatic Ecosystems, Gulf Breeze Lab, Pensacola, Florida, 9-14 April 1978.

-Bergman

EPA-DOE-HEW Workshop on Priorities for Environmental Research Needs related to Oil Shale Development, Denver, Colorado, 16-21 April 1978.

-Bergman

Annual Meeting of the American Society of Limnology and Oceanography, University of Victoria, Victoria, British Columbia, 19-22 June 1978.

-Parker, Fannin, Marcus

Annual Meeting of the American Fisheries Society, University of Rhode Island, Kingston, 21-25 August 1978.

-Bergman, DeGraeve, Geiger, Johnson

First Life Sciences Symposium on Potential Health and Environmental Effects of Synthetic Fossil Fuel Technologies--DOE/ORNL. Gatlinburg, Tennessee, 25-28 September, 1978.

-Bergman

American Society for Testing and Materials--Aquatic Toxicology Section. Annual Meetings, New Orleans, Louisiana 17-18 October 1978.

-DeGraeve, Lebsack

5.4 Papers Submitted or Presented

5.4.1 Papers Submitted but Not Yet Presented

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

5.4.2 Papers Presented

"Effects of oil shale retort water on rainbow trout." A. D. Anderson, M. E. Lebsack, D. S. Farrier and R. E. Poulson. Presented at American Chemical Society Annual Meeting, 12-17 March 1978 (copy of abstract presented in Annual Progress Report, 15 May - 15 December 1978).

"A simple screening system to identify environmentally persistent hydrocarbons." T. E. Fannin, H. L. Bergman, M. Parker, M. D. Marcus, J. S. Meyer. American Society of Limnology and Oceanography Annual Meeting, 19-22 June 1978 (copy of abstract presented in Quarterly Progress Report, 5 December 1977 to 5 March 1978).

"Acute and embryo-larval toxicity of in situ coal gasification condenser water and its constituents." G. M. DeGraeve, R. L. Overcast and H. L. Bergman. American Fisheries Society Annual Meeting, 21-25 August 1978 (copy of abstract presented in Quarterly Progress Report, 5 December 1977 to 5 March 1978).

"A transmission electron microscopy study of gill histopathology in rainbow trout and fathead minnows exposed to coal gasification process waters." R. D. Johnson and H. L. Bergman. American Fisheries Society Annual Meeting, 21-25 August 1978 (copy of abstract presented in Quarterly Progress Report, 5 March to 5 June 1978).

"Effects of complex effluents from in situ fossil fuel processing on aquatic biota." H. L. Bergman, G. M. DeGraeve, A. D. Anderson and D. S. Farrier. Presented at First Life Sciences Symposium and to be published in Symposium Proceedings (see 5.3 above and 5.5.3 below).

5.5 Publications

5.5.1 In Preparation

"Toxicity of in situ coal gasification process water and major phenolic constituents to fathead minnows, 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)--Editor: Marcus.

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

"Sublethal effects of in situ oil shale process water on rainbow trout." (tentative title)--Lead authors: A. D. Anderson, Lebsack.

"Toxicity of an in situ oil shale process water to aquatic species." (tentative title)--Lead authors: A. D. Anderson, Lebsack.

"A modified factorial design for analysis of biodegradation of organic compounds." (tentative title)--Lead author: T. E. Fannin.

5.5.2 Papers Submitted

None which are not in press (see 5.5.3 below)

5.5.3 Papers in Press

Bergman, H. L., R. M. Carlson, C. W. Gehrs, M. Katz and M. L. Landolt. 1978. Phenol. p. 42-1 to 42-8. In: R. V. Thurston, R. C. Russo, C.M. Fetterolf, T. A. Edsall and Y. M. Barber, Jr. (eds.). Review of EPA Red Book: Quality criteria for water. Water Quality Section, American Fisheries Society, Bethesda, MD (In Press).

DeGraeve, G. M., W. J. Blogoslawski, W. A. Brungs, J. A. Fava, B. J. Finlayson, T. P. Frost, T. M. Krischan, J. M. Meldrim, D. T. Michaud, R. E. Nokatani and G. L. Seegert. 1978. Chlorine. p. 9-1 to 9-9. In: R. V. Thurston, R. C. Russo, C. M. Fetterolf, T. A. Edsall and Y. M. Barber, Jr. (eds.). Review of EPA Red Book: Quality criteria for water. Water Quality Section, American Fisheries Society, Bethesda, MD (In Press).

Bergman, H. L., G. M. DeGraeve, A. D. Anderson and D. S. Farrier. 1979. The effects of complex effluents from in situ fossil fuel processing on aquatic biota. Proceedings of the First Life Sciences Symposium on Potential Health and Environmental Effects of Synthetic Fossil Fuel Technologies, U.S. Department of Energy-Oak Ridge National Laboratory, September 1978 (In Press).

5.5.4 Papers Published

None during 1978.

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Barbour, F. A., F. D. Guffey and S. M. Dorrence. 1978. Preliminary examination of waste waters produced during an in situ retorting of tar sands. p. 743-749 In: D. A. Redford and A. G. Winestock (eds.). The oil sands of Canada and Venezuela - 1977. The Canada Institute of Mining and Metallurgy Spec. Pub. 17.

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