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ENVIRONMENTAL QUALITY RESEARCH FISH AND AUFWUCHS BIOASSAY

Fourth Annual Report

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TECHNICAL REVIEW AND APPROVAL

AMRL-TR-79-70

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

This report contains the results of research efforts of a project concerned with defining the effects of potential environmental contamination resulting from the use of certain Air Force materials on fresh water and saline water fish. Materials being evaluated include JP-4, JP-8, and the hydrazines. Techniques for exposing organisms to these substances are discussed and results of such exposures are presented.

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SUMMARY AND CONCLUSIONS

This report deals with the following topics:

The toxicity of the jet fuels JP-8 and JP-4 to rainbow trout (Salmo gairdneri).

The toxicity of the rocket fuel components, hydrazine (H), 1, 1-dimethylhydrazine (UDMH), and 1-methylhydrazine (MMH) to the three-spine stickleback (Gasterosteus aculeatus), the Bay mussel (Mytilus edulis), the crab species (Hemigrapsus nudus), and to aufwuchs.

A discussion of the relative toxicity and environmental impact of the jet fuels JP-4, JP-8, and JP-9.

A discussion of the relative toxicity and environmental impact of the hydrazines.

The use of the purge-and-trap method for quantitation of JP-4.

Results of two continuous-flow, partial chronic bioassays (a high concentration range study of 88 days duration and a low concentration range study of 112 days duration) of the water soluble fraction (WSF) of JP-4 to rainbow trout supported the following conclusions:

There was no significant effect on the success of egg hatching in the range of concentrations examined (0-6. 1 ± 0. 4 mg/ℓ WSF of JP-4.

At WSF of JP-4 concentrations greater than 4. 5 ± 0. 9 mg/ℓ, all newly hatched fry died within 2 weeks after swim-up.

At all WSF of JP-4 concentrations examined (0. 5 ± 0. 2-6. 1 ± 0. 4 mg/ℓ), egg hatching rate was accelerated.

At WSF of JP-4 concentrations between 1. 0 ± 0. 5 and 1. 7 ± 0. 8 mg/ℓ there was a significant effect on rainbow trout survival and growth.

At the lowest WSF of JP-4 concentration examined (0. 5 ± 0. 2 mg/ℓ during the egg basket period; 0. 2 ± 0. 1 mg/ℓ during the open tank period) there was possibly an effect on growth; however, statistical analysis of results has not yet been completed to assess the significance of this effect.

WSF of JP-4 accumulation in whole body tissue increased with time of exposure and with aqueous WSF of fuel concentration. The average accumulation ratio was constant and equal to approximately 170.

In the first bioassay, quantitative analysis of WSF of JP-4 by the pentane extraction method indicated that the 100% WSF of JP-4 concentration (fuel retention time, 24 hr) was 27. 13 ± 8. 61 mg/ℓ. When the fuel retention time was 7 days, the mean WSF of JP-4 concentration decreased to 14. 92 ± 0. 97 mg/ℓ.

In the second bioassay utilizing twice weekly fuel replenishment, quantitative analysis of WSF of JP-4 by the purge-and-trap method indicated the mean of 1-day samples was 22.94 ± 3.1 mg/ℓ while the mean of 4-day samples was 18.16 ± 3.8 mg/ℓ.

The recovery of WSF of JP-4 by the quantitative purge-and-trap method was estimated to be 99%, compared to a recovery of 45% by the pentane extraction method.

Results of a continuous-flow partial chronic bioassay (112 days duration) of the WSF of JP-8 to rainbow trout supported the following conclusions:

There was no effect on the success of egg hatching in the range of WSF of JP-8 concentrations examined (0-8.0 ± 0.8 mg/ℓ).

The rate of egg hatching was accelerated at WSF of JP-8 concentrations greater than 1.5 ± 0.3 mg/ℓ.

At 8 mg/ℓ WSF of JP-8 no fish survived the alevin stage.

There was no effect on fish mortality at a WSF of JP-8 concentration of 1.5 ± 0.3 mg/ℓ.

At WSF of JP-8 concentrations of 2.1 ± 0.3 to 8.0 ± 0.8 mg/ℓ, there was a significant effect on fish mortality.

At all WSF of JP-8 concentrations examined (1.5 ± 0.3 to 8.0 ± 0.8 mg/ℓ) there was a significant effect on growth.

Accumulation of WSF of JP-8 in trout whole body tissue was not proportional to aqueous WSF of JP-8 concentration.

The accumulation ratio was in the range 63-112.

Results of a 21-day continuous flow bioassay of hydrazine to aufwuchs indicated the following conclusions:

A concentration of 0.065 mg/ℓ hydrazine stimulates the growth of benthic biomass.

A concentration of 0.17 hydrazine has no effect on the growth of benthic biomass.

A concentration of 0.5 mg/ℓ hydrazine represses both growth and photosynthetic oxygen production of benthic biomass.

Results of continuous-flow bioassays of MMH to stickleback, mussels, crabs, and aufwuchs supported the following conclusions:

The 336-hr LC50 for crabs and mussels was between 0.012 and 0.15 mg/ℓ MMH.

The estimated 336-hr LC50 value for stickleback was 0.011 mg/ℓ MMH (summer study).

A winter study indicated that the 336-hr LC50 for MMH to stickleback was between 0.07 and 0.26 mg/ℓ.

The difference in LC50s of the two studies was attributed to the lower temperature (8°C) of the winter study compared to the 18°C temperature of the summer study and to the effect of a high silt content on fish mortality during the summer study.

The no effect level for photosynthetic activity of aufwuchs appears to be between 0.012-0.15 mg/ℓ MMH.

For aufwuchs growth, the no effect level appears lower – between 0–0.012 mg/ℓ.

Results of continuous-flow bioassays of UDMH to stickleback, mussel, crabs, and aufwuchs supported the following conclusions:

The 336-hr LC50 for stickleback is 0.22 mg/ℓ UDMH.

UDMH concentrations of 0.18 mg/ℓ permitted 100% survival of mussels and crabs; at the next highest concentration examined (0.46 mg/ℓ UDMH) there was 100% mortality of mussels and crabs.

Mussels were slightly more sensitive than crabs to UDMH based on exposure time–survival data.

Aufwuchs growth and activity were adversely affected by UDMH at all levels examined (>0.18 mg/ℓ).

PREFACE

The research reported herein was conducted at the Sanitary Engineering Research Laboratory, University of California at Berkeley, under the terms of contract F33615-76-C5005 with the U. S. Air Force. The contract monitor was Lt. Col. C. B. Harrah, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio. Professors David Jenkins and Robert C. Cooper were the Principal Investigators. Mr. Stephen A. Klein was the project manager. Ms. P. C. Ulrichs and Mr. Robert Okazaki were responsible for the conduct of bioassays.

Mr. Francis Jenq, Mr. Steve Onysko, Mr. Mark Knox, Ms. Bonnie Jones, Ms. Elahé Ensanni, Ms. Nicole Houel, and Ms. Elyse Heilshorn, candidates for the M. S. degree in Sanitary Engineering, served as research assistants.

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INTRODUCTION

Studies included in this report are directed toward providing information on the toxicity to aquatic life of the kerosene-based jet fuels, JP-4 and JP-8, and the rocket fuel components hydrazine, monomethylhydrazine (MMH) and unsymmetrical dimethylhydrazine (UDMH). These materials are currently in use by the U.S. Air Force.

To define the environmental impact of a toxicant on aquatic life, an experimental protocol has been established. This protocol for jet fuels includes conducting acute static, acute continuous-flow, and chronic continuous-flow bioassays on warm-water and cold-water fish. A chronic continuous-flow study of 4-months duration on the effect of the water-soluble fraction (WSF) of JP-8 on the cold-water fish, rainbow trout (Salmo gairdneri), is reported herein. Two chronic continuous-flow studies of 3.5 months duration, each on the effect of the WSF of JP-4 on rainbow trout, are also reported. These studies commenced with "eyed" eggs and examined the hatching, the growth, and development of fry and concluded with analysis of fish tissue for fuel accumulation.

Studies on rocket fuel components have been conducted in saline water from San Francisco Bay at the large-scale bioassay facility ("analog" facility) of the Sanitary Engineering Research Laboratory. The experimental protocol includes performing acute static, acute "spill," and 14-day continuous-flow bioassays on the 3-spine stickleback (Gasterosteus aculeatus) and aufwuchs (attached periphyton growth). In some of these studies, the Bay mussel (Mytilus edulis) and the mud-flat crab (Hemigrapsus oregonensis) were also examined. "Spill" studies were reported previously on the three rocket fuel components. Reported herein are 14-day continuous-flow studies on the toxicity of MMH and UDMH. A similar study with hydrazine was previously reported, but a repeat study on the aufwuchs portion of the study is included herein.

MATERIALS AND METHODS

INTRODUCTION

Materials and methods have been detailed in previous reports (November 1977 Second Annual Report AMRL-TR-77-54 and November 1978 Third Annual Report AMRL-TR-78-65) and will not be repeated here. Only new techniques that differ from those presented previously will be discussed.

QUANTITATIVE ANALYSIS OF WSF OF JP-8

WSF of JP-8 was analyzed quantitatively by pentane extraction, evaporation, and gas chromatography. Analytical data had to be corrected by a factor of 1.5032 to account for losses during rotary evaporation.

Analytical precision at an average WSF of JP-8 concentration of 10.47 mg/ℓ was 0.38 mg/ℓ (Appendix 1).

EFFECT OF RESIDENCE TIME OF FUEL IN FUEL SOLUBILIZER ON WSF OF JP-8 CONCENTRATION

As noted in previous reports, the concentration of WSF of fuel produced by the solubilizer varies with the residence time of the fuel in the solubilizer. Typically, the solubilizer is replenished with fresh fuel once per week. The product WSF gradually decreases in concentration during this period. The magnitude of the concentration variation was measured by taking duplicate samples of solubilizer effluent, 18 hr and 168 hr after fuel replenishment. The mean concentration of the 18-hr samples was 10.92 ± 0.87 mg WSF of JP-8/ℓ, and the mean concentration of the 168-hr samples was 9.68 ± 0.30 mg WSF of JP-8/ℓ. The 1.24 mg/ℓ decrease between fuel replenishments represents an approximate 11% decrease in WSF of JP-8 concentration.

EFFECT OF RESIDENCE TIME OF FUEL IN FUEL SOLUBILIZER ON QUANTITATIVE COMPOSITION OF WSF OF JP-8

Using the purge-and-trap apparatus followed by GC analysis, 18-hr and 168-hr samples of WSF of JP-8 produced by the solubilizer were analyzed.

Typical chromatograms are presented in Figure 1, which shows in addition to the solubilizer product, chromatograms of a 14% dilution of WSF of JP-8 from one of the exposure tanks. A pronounced decrease occurs in one of the peaks (retention time – 280 sec) between the 18-hr and 168-hr samples. Analysis of 6 sets of samples showed that the 280-sec peak decreased from a mean of 5.3% (± 1.69%) of the total WSF of JP-8 peak area at 18 hr to a mean of 0.92% (± 0.69%) at 168 hr. This relative decrease is 7.5 times greater than the overall 11% decrease in total peak area noted over the same time period. In addition to the 280-sec peak, other peaks in the 0-1000 sec retention time range appear to deplete as the fuel retention time in the solubilizer increases.

JP-4 QUANTITATION

In the first rainbow trout bioassay, the pentane extraction method was used and the fuel in the solubilizer was replenished once per week. The mean 100% WSF of JP-4 concentration (fuel retention time, 24 hr) was 27.13 ± 8.61 mg/ℓ (n = 5). When the fuel retention time was 7 days, the mean WSF of JP-4 concentration decreased to 14.92 ± 0.97 mg/ℓ (n = 5).

This almost twofold variation in concentration was unacceptable. Therefore, in the second rainbow trout bioassay the fuel in the solubilizer was replenished twice per week. The purge-and-trap method was used for quantitative analysis of JP-4. Results of these analyses are presented in Appendix 2. The mean of 19 samples of WSF of JP-4 produced from the solubilizer containing fuel with a range of detention times from 1-4 days was 20.64 ± 1.8 mg/ℓ. The effect of fuel age on WSF of JP-4 concentration is further illustrated by these data – the mean of the 1-day samples was 22.94 ± 3.1 mg/ℓ while the mean of 4-day samples was 18.66 ±3.8 mg/ℓ.

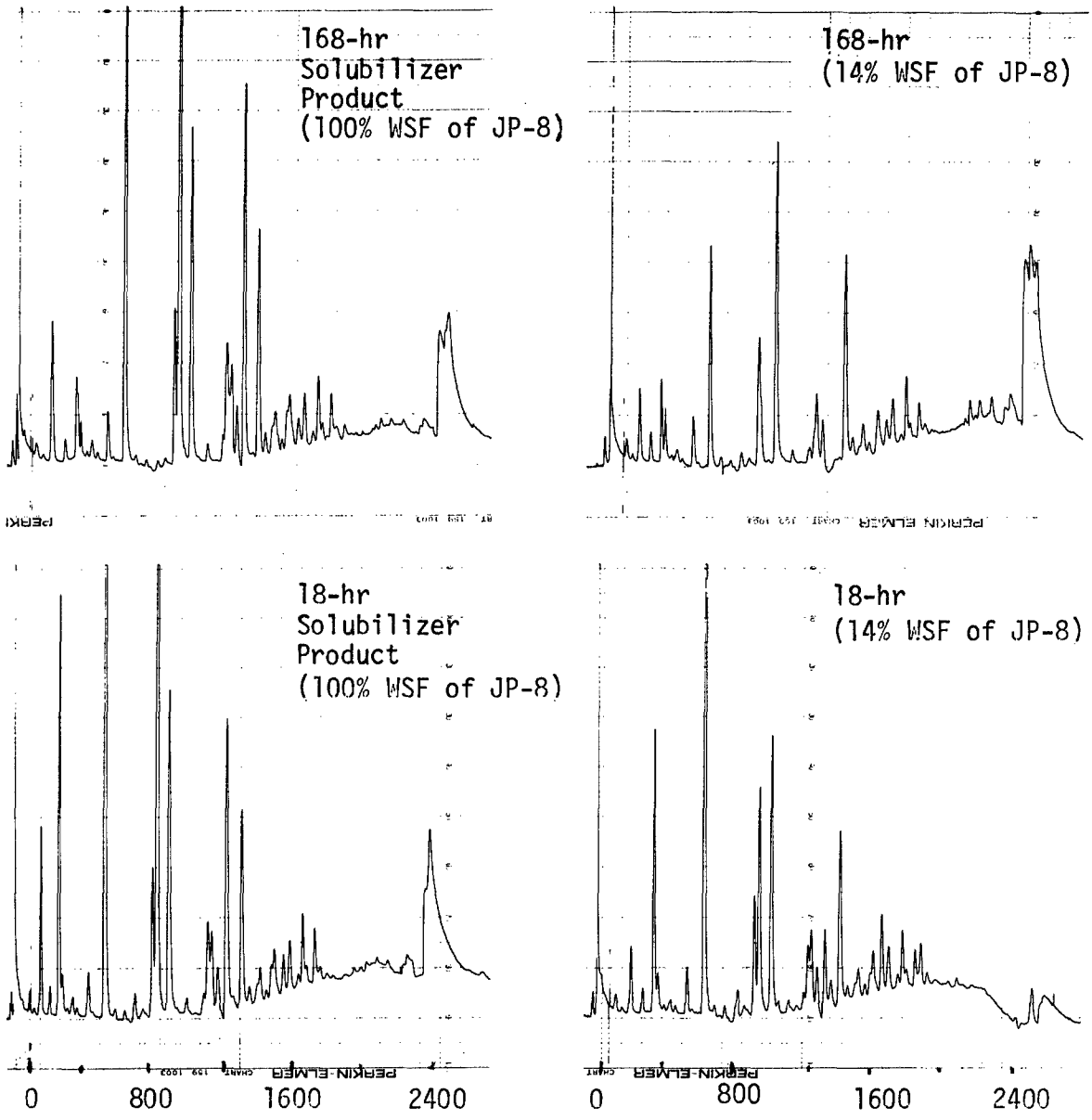


FIGURE 1. PREFERENTIAL DEPLETION OF COMPONENTS OF WSF OF JP-8 WITH FUEL RETENTION TIME IN SOLUBILIZER

RECOVERY ACHIEVED BY PURGE-AND-TRAP METHOD FOR QUANTITATIVE ANALYSIS OF JP-4

A sample of WSF of JP-4 was double extracted into pentane, the pentane volume reduced to 3 mℓ, and a 2 μℓ aliquot injected into the GC. Based on the 100-fold reduction, the aliquot contained the equivalent of 200 μℓ of the original aqueous sample. A 200-μℓ aliquot of the original sample was analyzed by the purge-and-trap method. Three samples were analyzed. Sample No. 1 was analyzed by standard isothermal techniques: GC column at 160°C for both pentane extraction injection and purge-and-trap. In the purge-and-trap analysis, the GC column was maintained at room temperature during sample desorption and then the GC oven temperature was immediately increased to 160°C. Sample No. 2 (fuel age, 96 hr) and Sample No. 3 (fuel age, 24 hr) were analyzed by the same method used for Sample No. 1 except that a 10-min initial delay at room temperature before increasing the GC oven temperature to 160°C was used.

Figure 2 depicts the chromatograms of Sample No. 1 obtained by the standard quantitation procedure using isothermal operation. In the pentane extraction method, the huge pentane solvent peak masks compounds $< C_8$ and volatility losses during rotary evaporation greatly reduce recovery of compounds up to C_{11}. For the purge-and-trap method, there is poor recovery of $> C_{11}$ compounds. The magnitude of loss in recovery was examined by total peak area measurements (Table 1).

TABLE 1

COMPARISON OF PEAK AREAS BY PURGE-AND-TRAP AND PENTANE EXTRACTION METHODS

(Isothermal Operation, WSF of JP-4, Sample No. 1)

Zone	Peak Area MV.sec		
	Purge-and-Trap	Pentane Extraction	Difference
Through C_{11}	2,340,000	1,420,000	+920,000
Above C_{11}	76,200	97,100	-20,900
Total	2,420,000	1,520,000	+900,000

\+ indicates purge-and-trap peak area > pentane extraction peak area
\- indicates pentane extraction peak area > purge-and-trap peak area

In the analysis of Samples 2 and 3, the 10-min delay at room temperature in the GC analysis spread out the early peaks (Figure 3) and permitted a closer examination of zones of the chromatogram – peak areas below C_8 (Zone 1), between C_8 and C_{11} (Zone 2) and above C_{11} (Zone 3). Comparison of the two chromatograms gives a clear picture of the extent that the pentane solvent peak masks the early JP-4 peaks and indicates the significant volatility loss caused by the pentane volume reduction step. Table 2 presents the peak area data for Samples 2 and 3 and shows that the greatest losses by

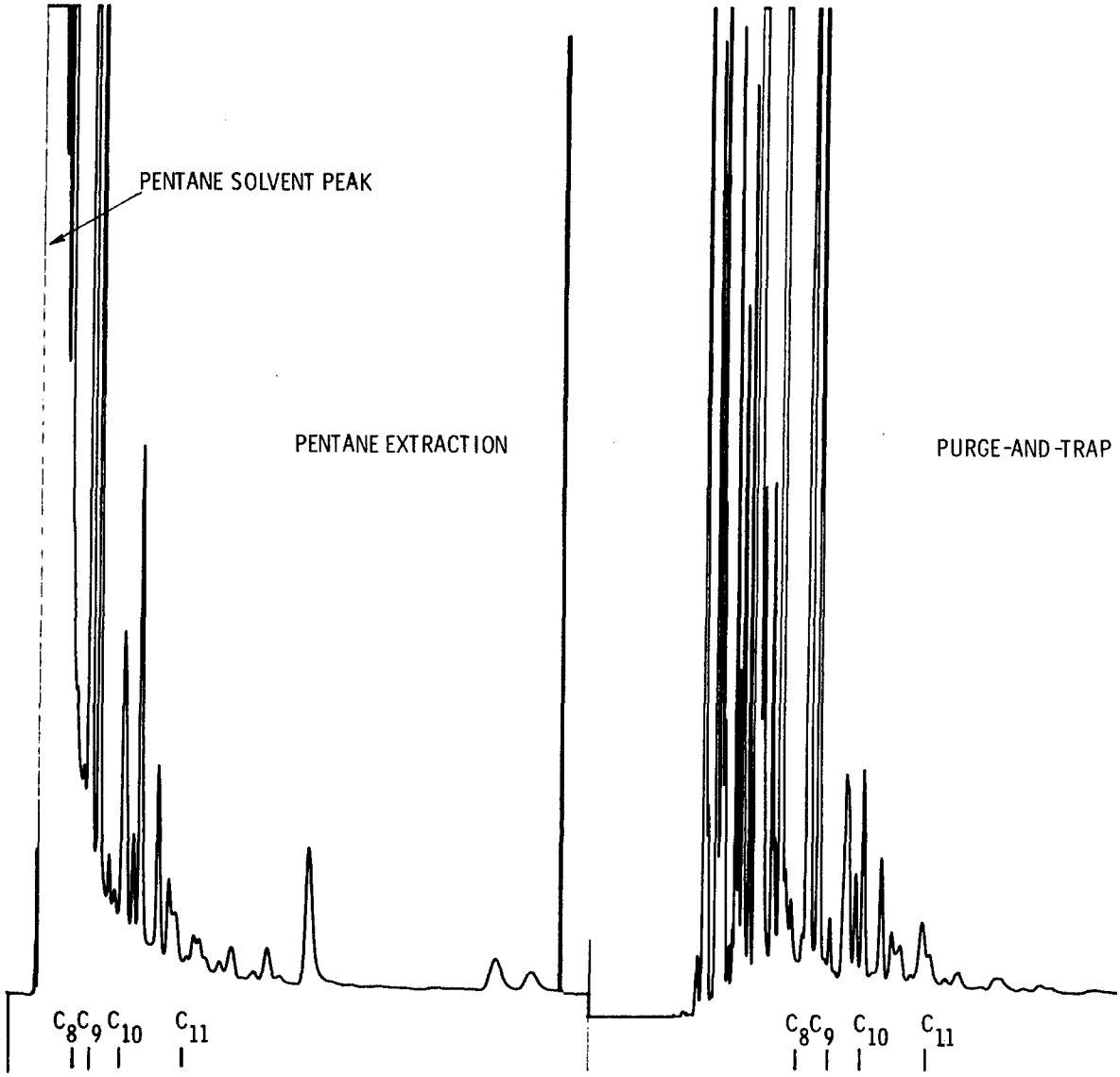
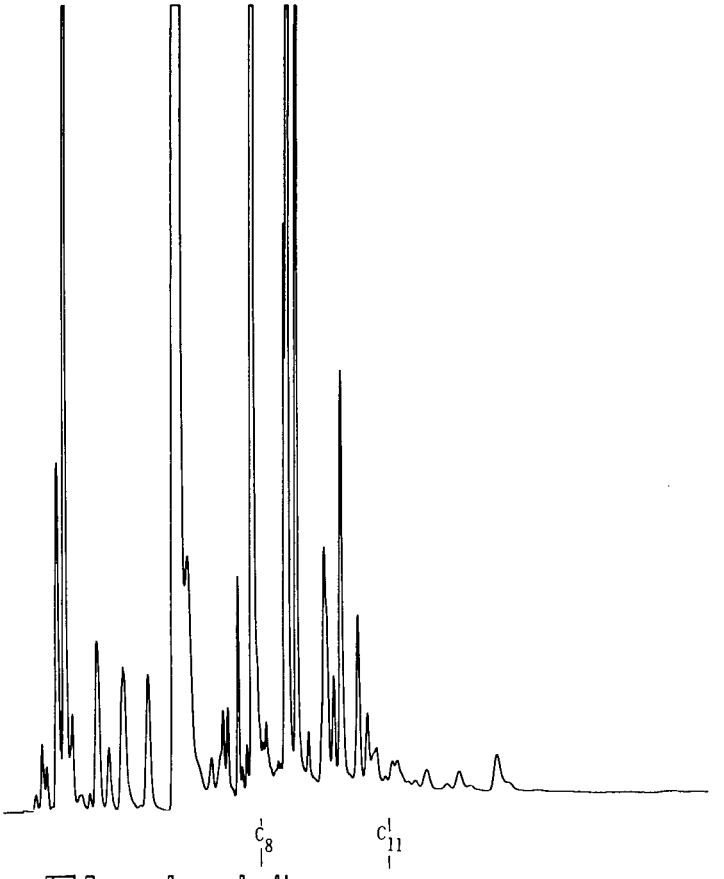


FIGURE 2. ANALYSIS OF WSF OF JP-4 COMPARISON BY PENTANE EXTRACTION AND PURGE-AND-TRAP METHODS



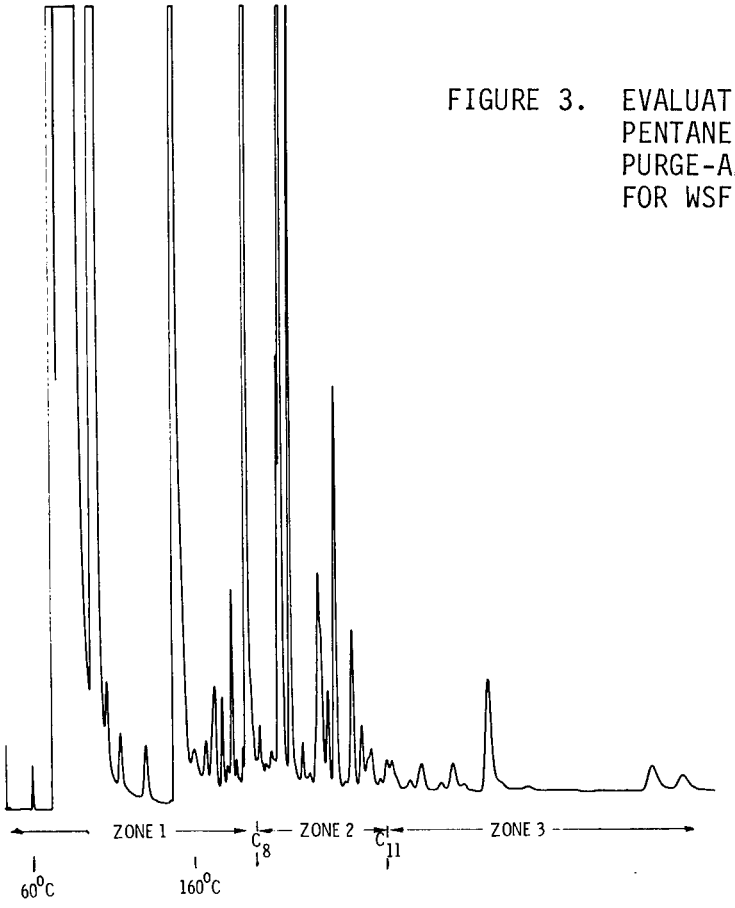


FIGURE 3. EVALUATION OF RECOVERY OF PENTANE EXTRACTION AND PURGE-AND-TRAP METHODS FOR WSF OF JP-4

the pentane extraction method occur below C_8. Both methods show virtually the same recovery above C_8. Using the same rationale as for Sample 1, the purge-and-trap method has a 98% recovery for WSF of JP-4. Because of this high recovery, no correction factor was applied to the GC data.

TABLE 2

COMPARISON OF PEAK AREAS BY PURGE-AND-TRAP AND PENTANE EXTRACTION METHODS

(Isothermal Operation after 10-min delay, WSF of JP-4,

Sample Nos. 2 and 3)

Sample No. 2

Zone	Peak Area MV.sec		
	Purge-and-Trap	Pentane Extraction	Difference
1 ($<C_8$)	1,280,000	711,000	+569,000
2 (C_8-C_{11})	499,000	505,000	-6,000
3 ($>C_{11}$)	81,000	126,000	-44,900
TOTAL	1,860,000	1,340,000	

Sample No. 3

Zone	Peak Area MV.sec		
	Purge-and-Trap	Pentane Extraction	Difference
1 ($<C_8$)	1,750,000	695,000	+1,060,000
2 (C_8-C_{11})	533,000	442,000	+91,000
3 ($>C_{11}$)	83,500	132,000	-48,500
TOTAL	2,366,500	1,269,000	

The purge-and-trap technique has been shown to recover 100% of n-alkanes of C_6-C_{10} and > 90% recovery of the C_{11} n-alkane. If one assumes that the pentane extraction method completely recovers the $>C_{11}$ fraction, then the overall recovery of the purge-and-trap method for WSF of JP-4 is estimated at 99%.

EGG BASKETS

Studies with rainbow trout eggs were initiated by placing "eyed" eggs into 8-mesh, 0.028 in. (0.07 cm) diameter stainless steel wire baskets 10 in. (25.4 cm) by 10 in. (25.4 cm) square and 4 in. (10.2 cm) deep. The baskets were suspended to a depth of 2 in. (5.0 cm) beneath the water surface in each continuous-flow exposure tank. The influent flows of WSF of fuel and dilution were directed into the egg basket to maintain the flow of water necessary for egg development.

WATER SUPPLY

Dechlorinated tap water (Table 3) was the source of water for the JP-8 study. For the two continuous-flow JP-4 experiments, the water supply was a well located on the Richmond Field Station. Use of well water provided many advantages over the tap water supply. It eliminated the need for a dechlorination apparatus and the possibility of toxicity to fish from inefficient dechlorination. Temperature control for cold water fish experiments was improved by the more constant delivery temperature of groundwater compared to the seasonal temperature fluctuations of surface water. It was possible to maintain temperature in the bioassay tanks at 13°C (the optimal temperature for rainbow trout egg hatching). This was 4°C lower than could be maintained

TABLE 3

TAP WATER CHARACTERISTICS FOR CONTINUOUS FLOW WSF OF JP-8 EXPERIMENTS

Parameter	Value
pH	8.5
conductivity	65 micromhos/cm
total solids	56 mg/ℓ
total dissolved solids	44 mg/ℓ
total hardness (as $CaCO_3$)	34 mg/ℓ
Na^+	3 mg/ℓ
K^+	1 mg/ℓ
Cl^-	6.4 mg/ℓ
Mg^{++}	3.0 mg/ℓ
Ca^{++}	10.5 mg/ℓ
F^-	0.5 mg/ℓ

during the hot summer months when using tap water. The average of 3 sets of analyses conducted (10/3/78, 11/6/78, and 12/4/78) is presented in Table 4. The results indicate a moderately hard water of good bacterial quality and free of heavy metal contamination.

TABLE 4

CHEMICAL ANALYSIS OF RICHMOND FIELD STATION WELL WATER

Parameter	Value, mg/ℓ unless specified
Ca	54
Mg	40
Fe total	0.6
Mn	0.01
Na	64
K	0.9
TDS	508
Total Alkalinity (as $CaCO_3$)	214
Cl	77
SO_4	123
F	0.2
NO_3	4.2
Ag	<0.001
As	0.001
Ba	<0.1
Cd	<0.001
Cr	<0.004
Cu	<0.05
Hg	<0.002
Pb	<0.01
Se	<0.001
Zn	<0.1
pH (pH units)	7.3
Spec. Cond., mhos/cm	940
Coliform, MPN/100 mℓ	<2
Fecal, MPN/100 mℓ	<2

PARTIAL CHRONIC BIOASSAY RAINBOW TROUT EXPOSED TO WSF OF JP-8

A long-term continuous-flow bioassay was conducted to determine the chronic toxicity of the WSF of JP-8 to rainbow trout. Toxicity was assessed by the effect of WSF on egg hatching, fry growth and development, fry survival, and fuel accumulation in whole body tissue.

PROCEDURE

Eyed eggs of rainbow trout, obtained from the American River Fish Hatchery, were placed in duplicate banks of stainless steel tanks (Bank A and Bank B) under conditions identical to those used in previous cold-water experiments. The tanks each contained volumes of 80 ℓ and received water at a rate of 220 mℓ/min (nominal residence time, 6 hr). The water was maintained at approximately 15°C; no aeration was provided.

Parameters measured daily were temperature and flow rates of solubilizer product and dilution water. The pH, DO, and WSF of JP-8 concentrations were measured weekly.

The feeding schedule was 8 times per day and maintenance involved daily removal of excess food, twice weekly cleaning of tank sides, and once weekly cleaning of tank contents by recirculating them through a filter.

The desired percent concentrations of the WSF of JP-8 were 0, 14, 24, 42, and 75. For the latter concentration, the flow rates were adjusted to deliver 85% WSF of JP-8, but it was assumed that volatility loss would diminish the WSF of JP-8 to a concentration equivalent of $\sim$ 75%. These percent concentrations corresponded to approximately the following WSF of JP-8 mass concentrations: 14%, 1.5 mg/ℓ; 24%, 2.1 mg/ℓ; 42%, 3.2 mg/ℓ; 85%, 7.9 mg/ℓ.

Weekly measurements were made of WSF of JP-8 levels in the exposure tanks. To account for the decrease in concentration of WSF as the fuel retention time in the solubilizer increased, sampling days were randomly selected.

During the period that alevin were contained in the fry chamber, samples were usually removed directly from the chamber (rather than from the exposure tank). Later, when fry were released to the open tank, samples were taken from near the tank center.

The results of 17 sets of JP-8 measurements are presented in Table 5. The mean solubilizer product WSF of JP-8 concentration was 10.6 $\pm$ 0.6 mg/ℓ. There was good replication of JP-8 concentrations (Table 5) between the two banks of tanks on an overall basis although on individual sampling days there were occasionally large differences between the replicate tanks. No JP-8 was ever detected in the controls.

The study was initiated by placing 140 eyed eggs each into 10 egg baskets. To control fungus, the eggs and hatched fry were initially treated

TABLE 5

JP-8 CONCENTRATIONS DURING CHRONIC RAINBOW TROUT BIOASSAY

(JP-8, mg/L)

Expt. Day	100% WSF of JP-8	Bank A Tanks				Bank B Tanks			
		14%	24%	42%	85%	14%	24%	42%	85%
-2	10.6	1.8	2.2	3.7	8.2	1.7	2.4	3.5	8.2
7	11.0	1.6	2.2	3.6	8.3	1.8	2.1	3.6	8.2
11	10.2	1.4	2.6	2.8	6.9	1.4	2.4	2.9	6.9
20	10.4	-	1.9	3.4	8.1	0.7	1.4	2.4	8.6
28	11.1	-	1.7	2.7	-	1.5	2.2	3.3	-
32	10.6	1.3	2.3	3.1	-	1.4	2.2	3.1	-
40	10.2	1.3	1.6	3.1	-	1.2	1.8	3.0	-
49	10.8	-	1.7	-	-	0.9	1.5	-	-
54	11.9	1.4	1.5	-	-	1.4	1.5	-	-
61	10.0	1.5	1.3	-	-	2.4	2.6	2.9	-
67	11.0	1.4	1.3	-	-	2.0	1.3	-	-
75	11.6	1.0	1.8	-	-	1.3	1.9	-	-
82	9.3	0.8	1.4	-	-	1.1	1.4	-	-
90	9.9	1.4	2.0	-	-	1.5	1.7	-	-
98	10.5	1.7	2.5	-	-	-	1.2	-	-
105	10.9	-	1.4	-	-	-	1.4	-	-
110	9.6	-	1.2	-	-	1.1	2.2	-	-
Mean ±s	10.6 ±0.6	1.4 ±0.3	1.8 ±0.4	3.2 ±0.4	7.9 ±0.7	1.4 ±0.4	1.8 ±0.4	3.1 ±0.4	8.0 ±0.8

with 1.0 mg Malachite Green/ℓ and then daily for 2 weeks with 5-min periods of exposure to 0.1 mg Malachite Green/ℓ. The treatment procedure involved lifting the egg basket out of the tanks and into a stainless steel tray, then adding the Malachite Green to the water in the tray.

The feeding procedure, including type of food and frequency of feeding, and the disease control procedure followed the recommendations of Leitritz (1976).

DIARY OF EVENTS

A diary of the major events and actions occurring during the course of the bioassay is presented in Table 6. The procedure differed from previous bioassays in that a large number of alevin were hatched in each exposure tank and then thinned periodically to avoid overcrowding as the fry matured.

TABLE 6

DIARY OF CONTINUOUS FLOW PARTIAL CHRONIC TROUT BIOASSAY

Day(s)	Action/Event
0	140 eyed eggs placed in egg baskets in each exposure tank
4	Egg hatching complete
12-76	Survival measurements
25	Swim-up: alevin complete egg sac utilization and swim-up to surface
26	Tanks 1, 2, and 3 thinned* to 95 fish. Start of length and weight measurements for growth and development phase.
46	Tanks 1, 2, and 3 thinned to 65 fish. Weight and length measurement.
60	Tanks 1, 2, and 3 thinned to 50 fish. Weight and length measurement.
70	Tanks 1, 2, and 3 thinned to 30 fish. Weight and length measurement. Unexplained fish mortality in exposure and control tanks. End of survival measurements.
76-112	Attempts to determine cause of mortality and rectify situation.
112	Weight and length measurement. Analysis of fish tissue for JP-8. Conclusion of experiments.

* Thinning conducted when dissolved oxygen concentration fell below 6.0 mg/ℓ. Thinning maintained fish loading at approximately 1 g/ℓ.

The need to thin was ascertained by DO measurement – where DO levels of < 6.5 mg/ℓ were observed fry were thinned to leave remaining 1 g fish/ℓ dilution water. Because of the high mortality in Tanks 4 and 5, thinning was not necessary. Tanks 1, 2, and 3 were thinned to approximately 95 (from ~ 130) on May 26, on Day 46 they were thinned to approximately 65, and on Day 60 to approximately 50 fish. On Day 76, the tanks were thinned to 30 fish. The advantage of this procedure was that the effects of various fuel concentrations on fry growth rate could be assessed by measuring the length and weight of the fish removed from the tanks. This avoided the difficulties inherent in the previous procedure of retaining fry in the chambers and using photography to measure fish length. Also, without thinning, fry chambers were difficult to keep clean and maintain adequate water flows so that low DO values and WSF of JP-8 levels different from the chamber contents were produced.

Survival measurements had to be terminated on Day 76 because of an unexplained (and unrectified) mortality in both controls and exposure tanks. The length and weight measurements on the surviving fish were carried on to the conclusion of the experiment on Day 112.

Egg hatching was complete after 4 days. While the extent of hatching (90-98%) was virtually the same in all exposure tanks and the controls, the hatching rate was progressively accelerated at WSF of JP-8 concentrations greater than approximately 1.5 mg/ℓ on Days 2 and 3 (Table 7). At 1.5 mg/ℓ the hatching rate was not significantly different from the control.

TABLE 7

RAINBOW TROUT EGG HATCHING IN WSF OF JP-8

WSF of JP-8 mg/ℓ	Cumulative Number of Eggs Hatched at: day 1	2	3	4	Hatching Success %
0	13	24	236	264	94.3
1.46 ± 0.29	6	13	235	263	93.9
2.12 ± 0.34	27	104	246	252	90.0
3.17 ± 0.42	41	105	253	255	91.1
7.95 ± 0.75	22	172	272	274	97.9

The pattern of mortality was virtually identical in the control and the exposure tanks containing 1.4 mg/ℓ WSF of JP-8 (Figure 4). At WSF of JP-8 concentrations between 1.8 mg/ℓ and 8.0 mg/ℓ mortality increased progressively (Figure 4). At WSF of JP-8 concentrations of 1.8 and 3.2 mg/ℓ a significant increase in mortality occurred on "swim-up" day (Day 26).

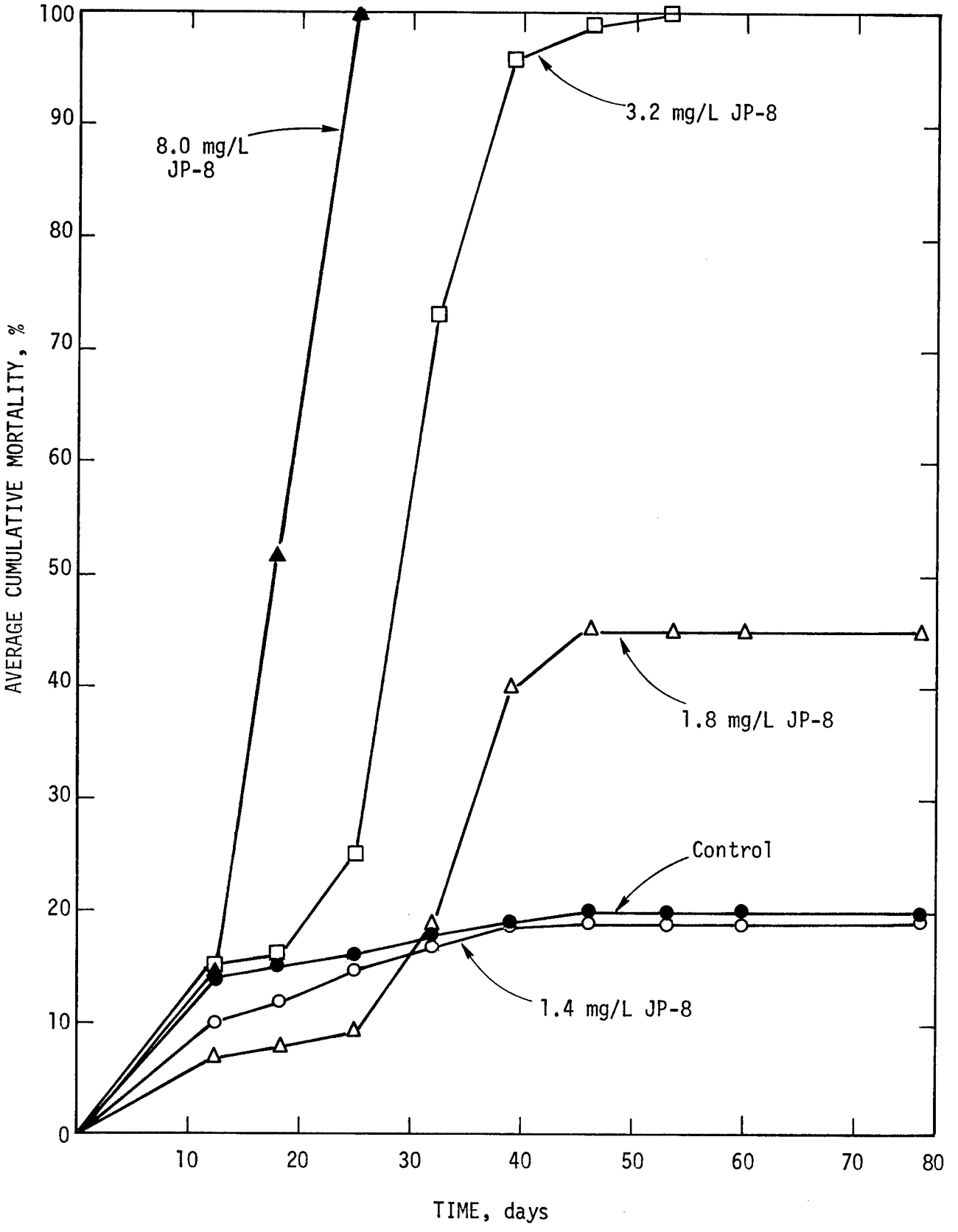


FIGURE 4. EFFECT OF WSF OF JP-8 ON RAINBOW TROUT SURVIVAL

TABLE 8

GROWTH RATE OF RAINBOW TROUT AS MEASURED BY LENGTH FROM SWIM-UP STAGE*, DAY 0, TO DAY 86

Tank	WSF of JP-8 mg/L ± s	Day 0 Length mm	Day 0 s** mm	Day 0 n***	Day 20 Length mm	Day 20 s mm	Day 20 n	Day 36 Length mm	Day 36 s mm	Day 36 n	Day 50 Length mm	Day 50 s mm	Day 50 n	Day 86 Length mm	Day 86 s mm	Day 86 n
Bank A																
1	0	23	0.1	17	33	0.3	20	41	0.6	13	50	0.4	18	72	0.9	19
2	1.4 ± 0.3	24	0.1	17	32	1.2	21	39	0.5	15	46	0.3	18	66	0.5	7
3	1.8 ± 0.4	24	0.1	8							39	0.3	16	58	0.5	19
4	3.2 ± 0.4	24	0.1	26												
Bank B																
1	0	24	0.1	12	32	0.4	21	44	0.5	20	50	0.5	19	74	0.5	13
2	1.4 ± 0.4	24	0.1	14	31	0.3	24	39	0.5	16	44	0.4	13	63	0.8	17
3	1.8 ± 0.4	24	0.1	23				33	0.4	20	37	0.3	22	54	0.4	22
4	3.1 ± 0.4	24	0.1	10												
Bank A + B																
1	0	24	0.1	29	32	0.3	41	43	0.5	33	50	0.4	37	73	0.8	32
2	1.4 ± 0.4	24	0.1	21	31	0.3	45	39	0.5	31	45	0.3	31	64	0.7	24
3	1.8 ± 0.4	24	0.1	31				33	0.4	20	38	0.3	38	56	0.5	41
4	3.2 ± 0.4	24	0.1	36												

* Swim-up stage, Day 0, corresponds to Day 26 of experiment

** s is standard deviation

*** n is number of fish measured.

Mortality reached a value of 45% in the 1.8 mg/ℓ exposure tank by Day 40; all fish died in 3.2 mg/ℓ by Day 53; in 8 mg/ℓ no fish survived the alevin stage.

The effect of WSF of JP-8 on the growth rate of fry as assessed by length and wet weight measurements is presented in Table 8 and Figures 5 and 6, respectively. Statistically significant differences (at the 99% level) in length between the fish exposed to 1.4 mg/ℓ WSF of JP-8 and the controls were first observed in one bank of tanks on Day 62. On days 76 and 112, these differences were significant in both banks of exposure tanks.

Fish wet weight was significantly different (the 95% level) between the controls and the lowest JP-8 concentration (1.4 mg/ℓ) after 46 days of exposure; by Day 76 these differences were significant at the 99% level.

Analysis of sacrificed fish at the end of the experiment (Day 112) for JP-8 showed (Table 9) that the accumulation of JP-8 in whole trout tissue was not proportional to aqueous JP-8 concentration (as had been the case for flagfish). Moreover, the concentration of JP-8 in trout (63-112 times that of the aqueous level) was less than for flagfish (159 times that of the aqueous level).

TABLE 9

WSF OF JP-8 ACCUMULATION IN WHOLE BODY TISSUE OF RAINBOW TROUT

WSF of JP-8 Concentration mg/ℓ ± s	WSF of JP-8 in Concentration Tissue mg/kg wet wt. ±s	Accumulation Ratio mg/kg wet wt. / mg/ℓ
1.4 ± 0.4	88 ± 25	63
1.8 ± 0.4	202 ± 34	112

PARTIAL CHRONIC BIOASSAYS – WSF OF JP-4 TO RAINBOW TROUT

HIGH RANGE OF JP-4 CONCENTRATIONS

Procedure

The bioassay was initiated with "eyed" eggs, obtained from the Mt. Lassen Trout Farm at Red Bluff, California. Egg baskets, each containing 173 eggs, were immersed to a depth of 2 in. (5.08 cm) in 80 ℓ liquid capacity continuous-flow bioassay tanks containing the following series of duplicate percentages of WSF of JP-4 (solubilizer product): 0, 12, 20, 32, and 50, corresponding respectively to WSF of JP-4 concentrations of 0, 1.7, 3.2, 4.5, and 6.1 mg/ℓ.

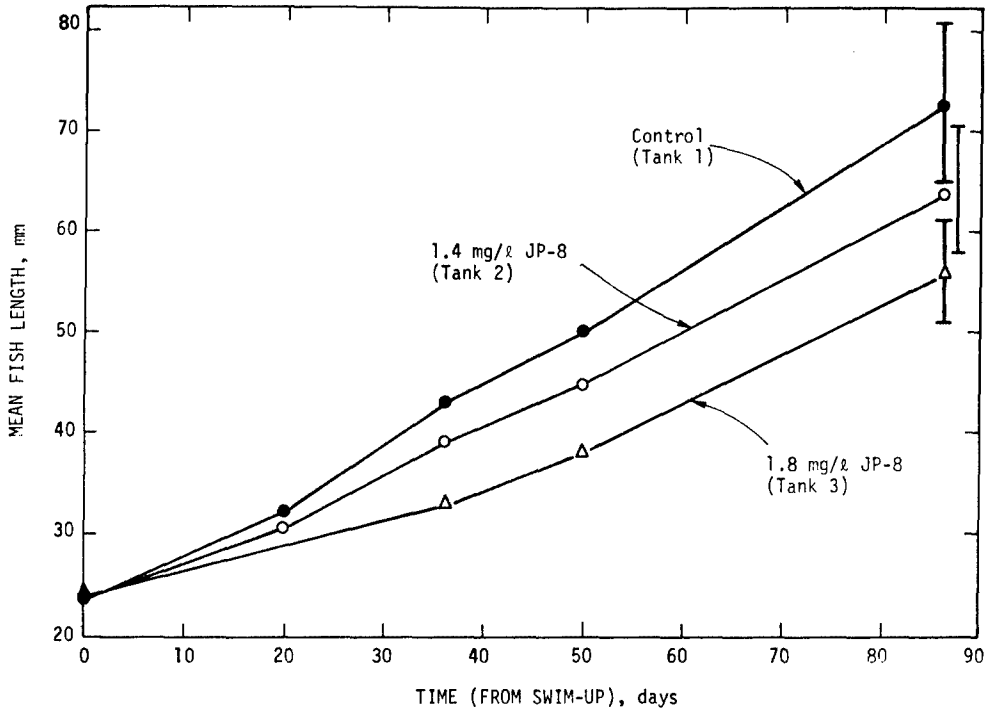


FIGURE 5. EFFECT OF WSF OF JP-8 ON LENGTH OF RAINBOW TROUT

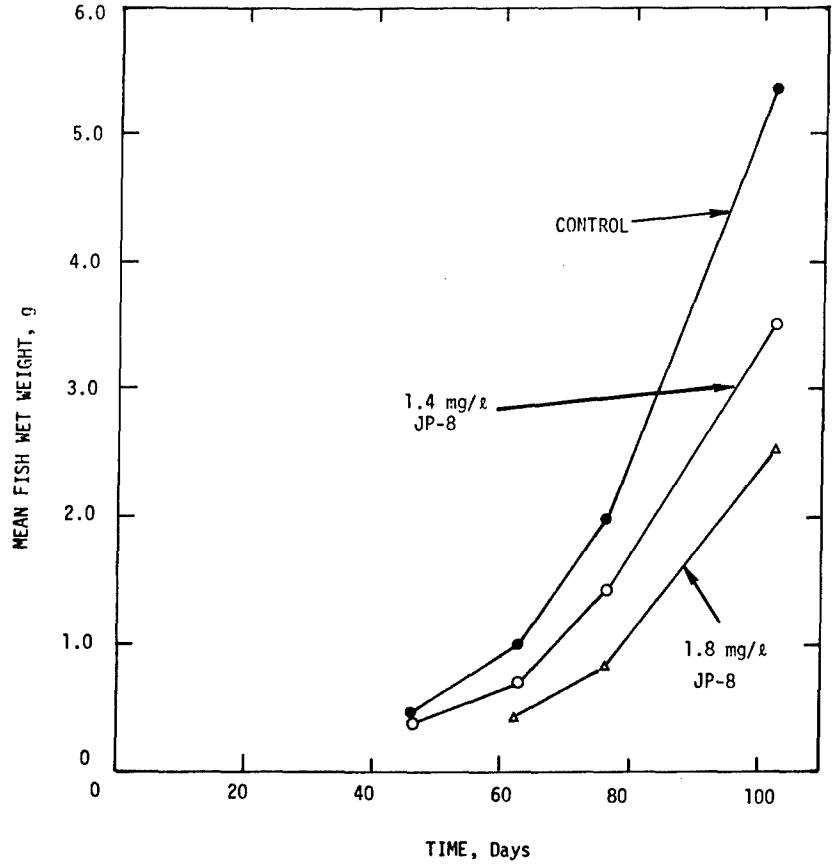


FIGURE 6. EFFECT OF WSF OF JP-8 ON WET WEIGHT OF RAINBOW TROUT

Water for solubilization and dilution was from a well located on the Richmond Field Station. With well water, the optimum temperature for hatching (13°C) could be maintained in the bioassay tank. Other than water source, the experimental procedure was the same as for the JP-8 bioassay with trout.

Hatching Results

Eggs hatched between Day 3 to Day 8 with a total hatching success in the range 86 to 96% which was unaffected by the WSF of JP-4 (Table 10).

TABLE 10

HATCHING OF RAINBOW TROUT EGGS IN WSF OF JP-4
(Average Values for Bank A and Bank B Tanks)

WSF of JP-4 Concentration:			Trout Hatched						
			Cumulative number on Day:						
Nominal %	mg/ℓ ±s	Original Number of Eggs	3	4	5	6	7	8	Total Hatched %
0	0	346	1	1	25	91	287	316	91
12	1.7 ± 0.8	346	0	3	34	85	270	298	86
20	3.2 ± 0.7	346	1	3	51	114	307	320	92
32	4.5 ± 0.9	346	0	14	135	188	318	326	94
50	6.1 ± 0.4	346	8	24	130	182	326	331	96

Although the total hatching success, measured after 8 days, was unaffected by the WSF of JP-4 concentrations tested, all levels of WSF of JP-4 accelerated hatching rate (Figure 7). The acceleration of hatching became more pronounced as the WSF of JP-4 concentration increased (Figure 7).

The effect of fuels on the hatching rate of fish eggs seems to be species related since both WSF of JP-8 and WSF of JP-4 accelerated hatching of rainbow trout eggs, but these two fuels retarded the hatching rate of flagfish eggs.

Survival

Swim-up took place on Day 25. Fish were thinned on Day 42 and again on Day 77. Average mortality data from the two banks of tanks are plotted in Figure 8. Statistical analysis of the mortality data showed that the two banks of exposure tanks were satisfactory replicates. The data are somewhat confounded by accidental mortalities that took place (1) on Day 16 in the Bank B, 4.5 mg/ℓ exposure tank and (2) between Days 46 and 49 in the 3.2 mg/ℓ exposure tanks. The first of these incidents was judged not to affect the mortality results to a significant degree because all of the fish in the 4.5 mg/ℓ exposure tank ultimately died. However, the accidental increase from 20 to 28% dilution of WSF of JP-4 that occurred in the second incident makes the mortality data of the 3.2 mg/ℓ exposure tank suspect. There was no statistically significant difference between survival of fish in the control and those exposed to 1.7 mg/ℓ WSF of JP-4.

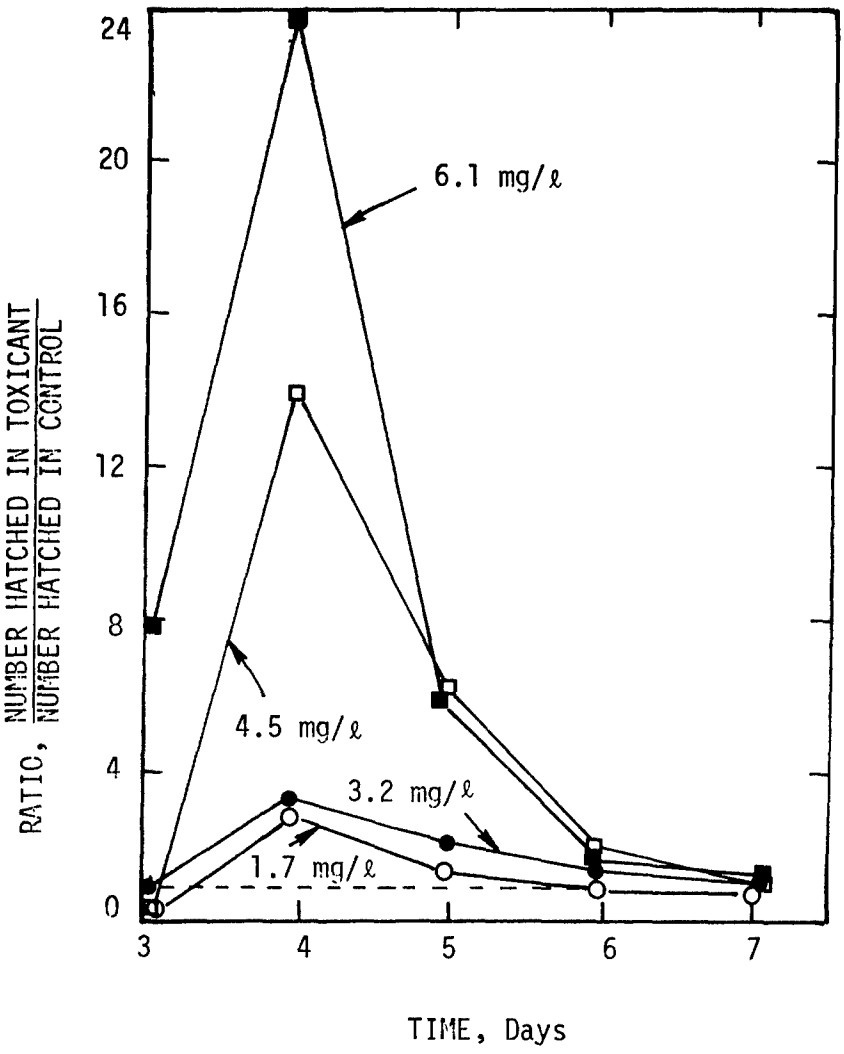


FIGURE 7. EFFECT OF WSF OF JP-4 CONCENTRATION ON RATE OF RAINBOW TROUT EGG HATCHING

Mortalities in the controls were significant. This has been ascribed to two factors: (1) damage that takes place early in the experiment when the eggs become trapped in the mesh of the egg baskets. This will be rectified in future series of bioassays by using a smaller mesh screen for egg baskets and (2) mechanical interference with fish during tank cleaning. This cause of mortality would be difficult to eliminate because tank cleaning is essential to maintain desired WSF of fuel concentrations and to eliminate infection. Transferring fish one by one to a separate tank would require an excessive amount of manpower.

Fish Lengths

Growth rates were determined by measurement of fish removed during thinning on Days 25 (swim-up), 42, 77, and at the end of the experiment (Day

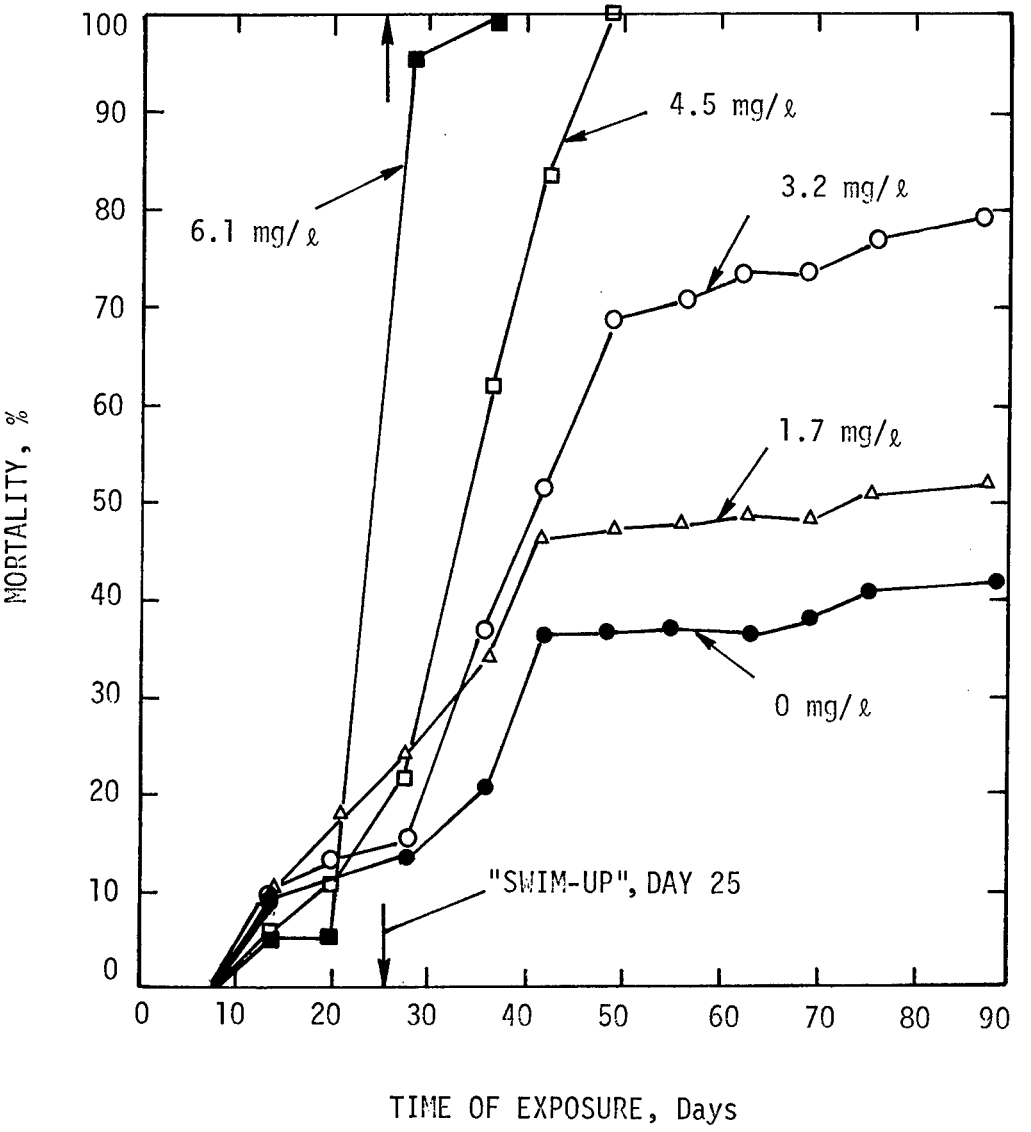


FIGURE 8. EFFECT OF WSF OF JP-4 ON SURVIVAL OF RAINBOW TROUT

88). There was a discernible and statistically significant reduction in length on the day of swim-up which was a function of WSF of JP-4 concentration and became more pronounced as the experiment progressed (Figure 9).

Fish Weights

The fish removed for length measurements were also weighed. Figure 10 indicates that the effect of even low WSF of JP-4 concentrations (1.7 mg/ℓ) was highly significant. By the termination of the experiment, there was a 42% decrease in fish weight at 1.7 mg/ℓ WSF of JP-4 and a 70% decrease at 3.5 mg/ℓ WSF of JP-4. Corresponding decreases in length were 16% and 36%, respectively.

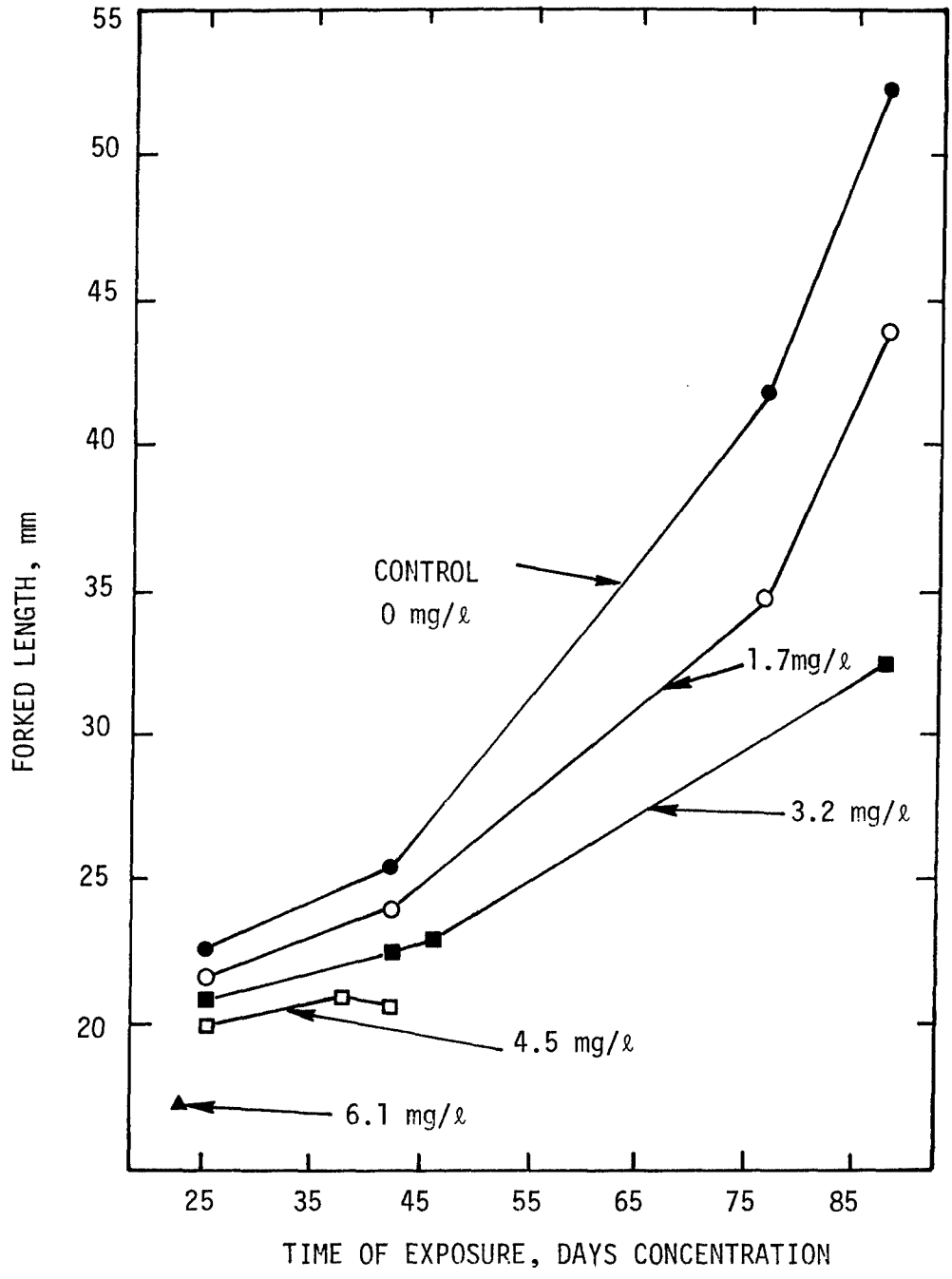


FIGURE 9. EFFECT OF WSF OF JP-4 ON AVERAGE RAINBOW TROUT FORKED LENGTH

Fuel Accumulation

Fuel accumulation in whole body tissue was measured on fish removed during thinning. Specific organ analysis could not be performed because the fish were too small to be dissected.

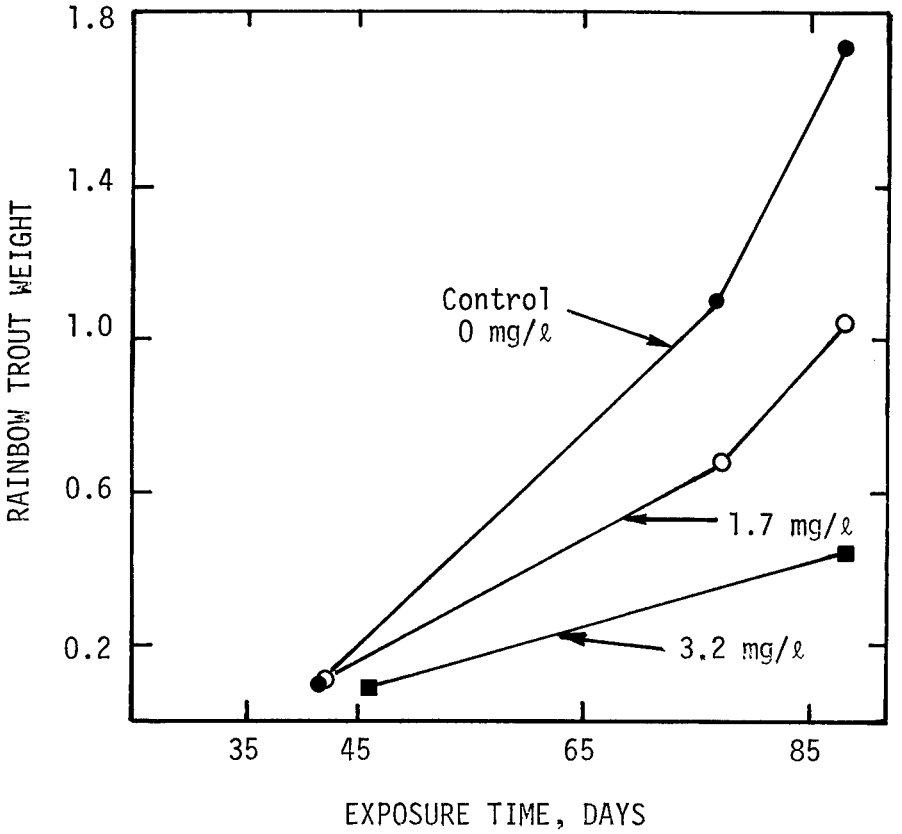


FIGURE 10. EFFECT OF WSF OF JP-4 ON RAINBOW TROUT WEIGHT (A BANK AND B BANK)

Accumulation increased with time of exposure and with aqueous fuel concentration. The average accumulation ratio was constant and equal to approximately 170.

Discussion

This experiment demonstrated that rainbow trout were extremely sensitive to JP-4. The results suggest that WSF of JP-4 is more toxic to trout than WSF of JP-8 (which indicated a no-effect level of 1.4 to 1.8 mg/ℓ). Because this experiment failed to detect the no-effect level of WSF of JP-4 on growth or survival, a further experiment was conducted at lower WSF of JP-4 concentrations.

LOW RANGE OF JP-4 CONCENTRATIONS

Procedure

The procedure was identical to the bioassay of the "high" JP-4 concentrations except that the fuel in the solubilizer was replenished twice instead of once per week. A total of 280 "eyed" eggs were placed in each egg basket and exposed in continuous-flow bioassay tanks to WSF of JP-4 levels of 0, 0.5, 1.1, 1.8, and 3.6 mg/ℓ prior to swim-up and WSF of JP-4 levels of 0, 0.3,

0.6, 1.1, and 1.8 mg/ℓ following swim up. The lower fuel levels were ascribed to (1) the presence of fish food which promoted bacterial growth in the tanks and thus increased fuel biodegradation and (2) higher volatility losses because of the increased turbulence in the tanks during the open-tank period of the bioassay.

Hatching Results

Eggs hatched predominantly between Day 3 and Day 8 with a total hatching success unaffected by any WSF of JP-4 concentration tested (Figure 11) and in the range 94 to 96%.

There was a significant acceleration of hatching at a WSF of JP-4 concentration of 3.6 mg/ℓ but not at concentrations below this (Figure 11). These results support those obtained in the previous bioassay and show that the acceleration of hatching of rainbow trout eggs by WSF of JP-4 is a reproducible phenomenon. It appears that WSF of JP-4 concentrations of less than approximately 2 mg/ℓ will not substantially affect hatching rate.

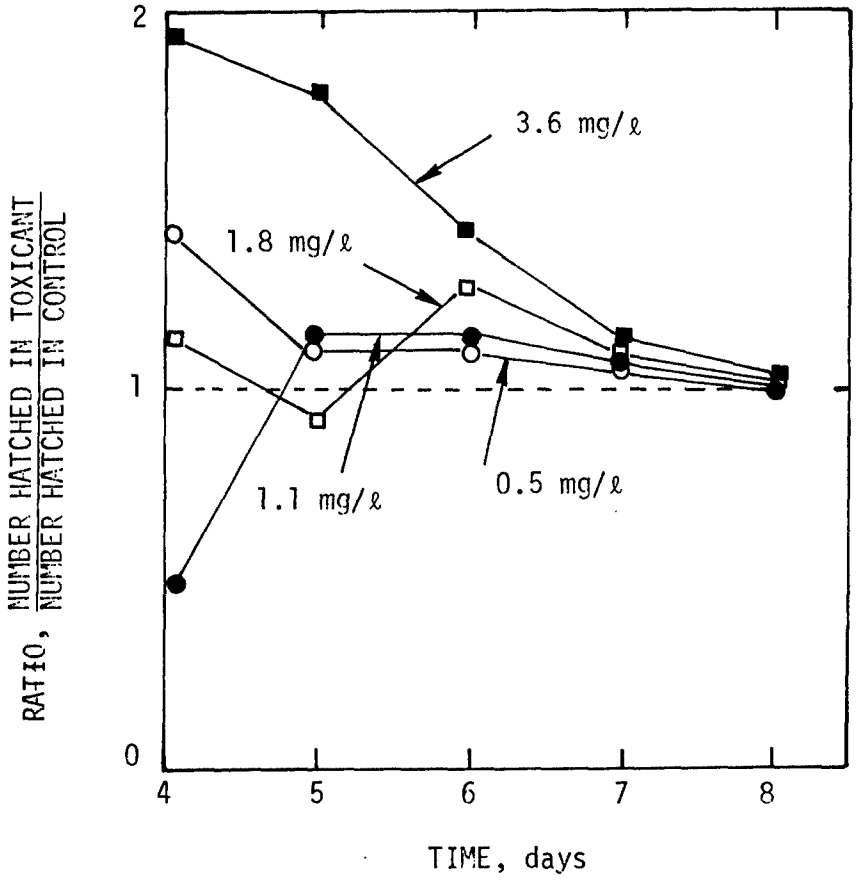


FIGURE 11. EFFECTS OF WSF OF JP-4 CONCENTRATION ON RATE OF RAINBOW TROUT EGG HATCHABILITY (2nd Bioassay)

Survival Results

Swim-up took place on Day 25. At that time, the fry in the Bank A tank containing 3.6 mg/ℓ WSF of JP-4 were accidentally lost. Half of the fry from the corresponding Bank B tank were transferred to make up for the loss. On Day 53, fry were accidentally lost in the Bank A 0.5 mg/ℓ and 1.1 mg/ℓ WSF of JP-4 tanks. Again, half of the fish from the corresponding Bank B tanks were transferred. Since the bioassay was designed to provide substantial thinning, these transfers did not deplete the number of fish below planned population levels. The population was thinned to approximately 200 on Day 25, to 170 on Day 45, to 85 on Day 53, to 55 on Day 84, and to 30 on Day 112.

Over the 112-day period of the bioassay, there were no deaths which could be attributed to WSF of JP-4 in the concentration range (0-3.6 mg/ℓ) tested (Figure 12). All deaths that occurred were from injuries caused by the daily cleaning of tanks.

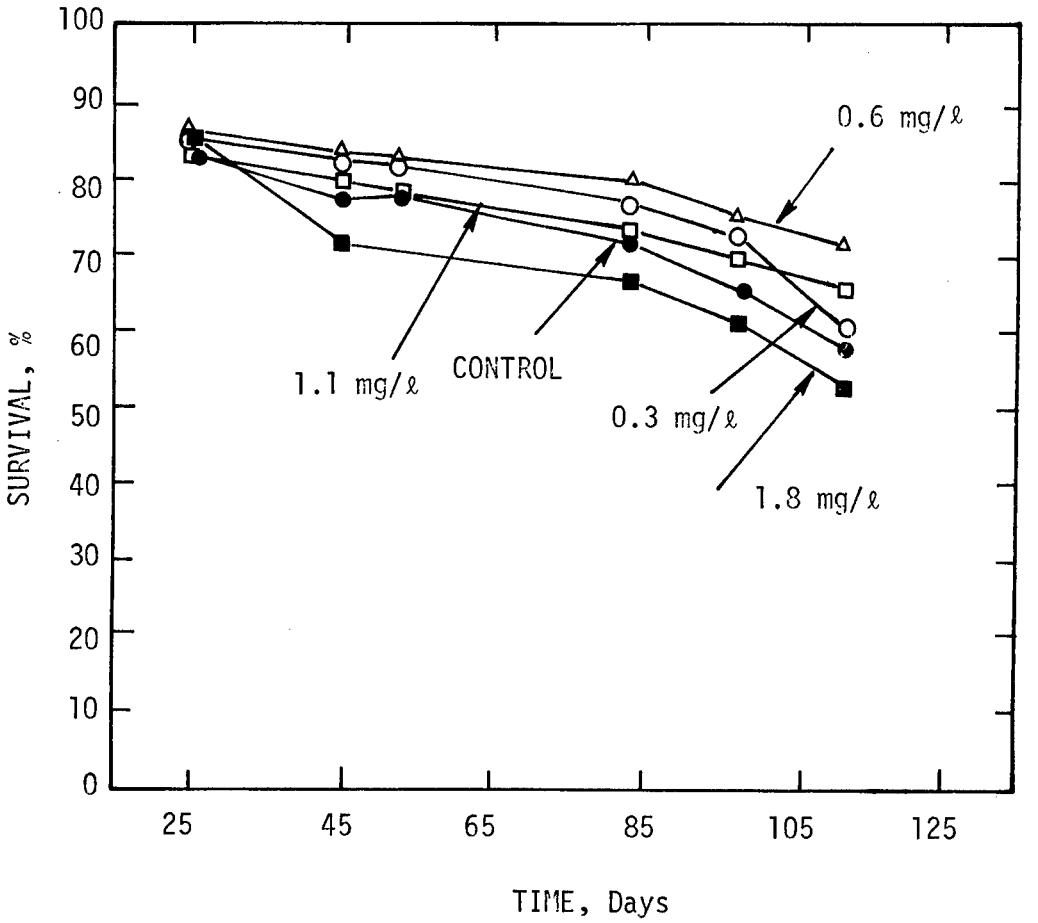


FIGURE 12. THE EFFECT OF JP-4 ON SURVIVAL IN RAINBOW TROUT (A + B BANKS)

Fish Lengths

The two banks of tanks were shown to be statistically identical and data from both banks were combined to give averages. Forked length was measured on fish removed during thinning and at the end of the experiment. Figure 13 shows that throughout the entire experimental period, the average length of fish exposed to WSF of JP-4 was less than the controls. The reduction in length, although consistent with time and WSF of JP-4 concentration, did not appear to be significant for WSF of JP-4 concentrations of 1.1 mg/ℓ or less. The average length of the fish exposed to 1.8 mg/ℓ WSF of JP-4 was significantly different from the controls for the length measurements made on Days 84, 98, and 112.

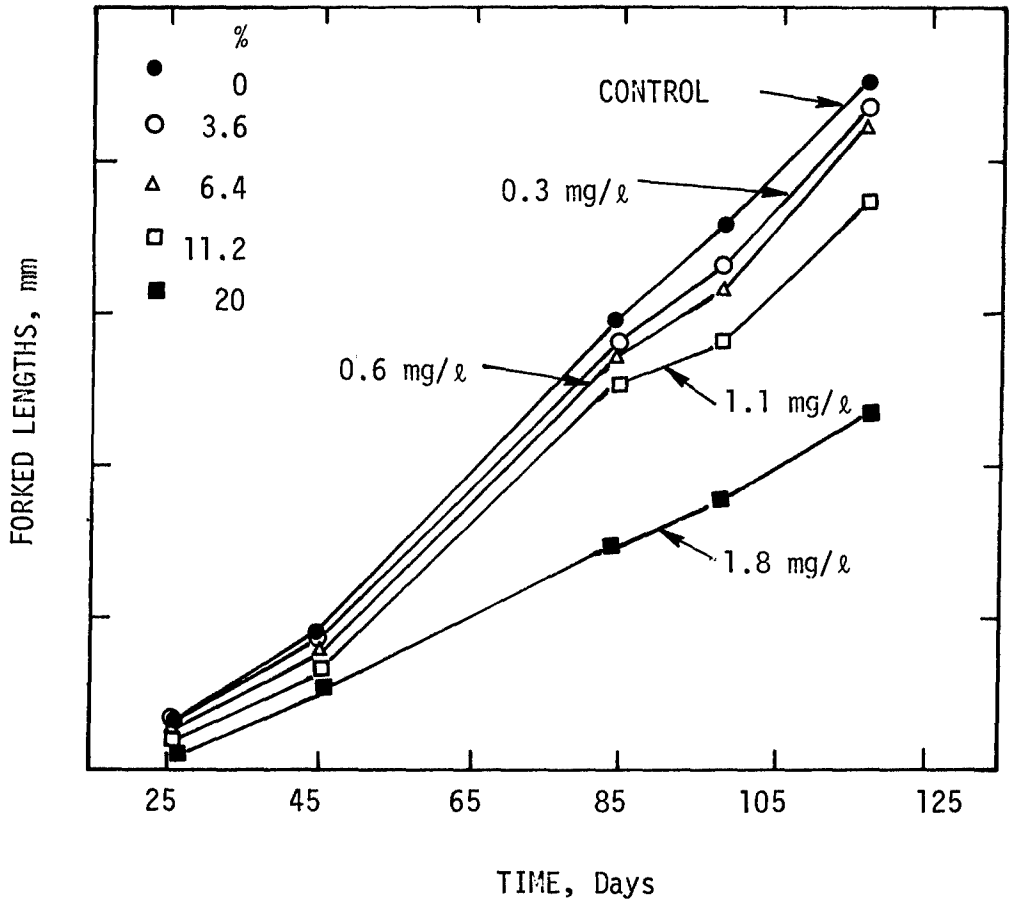


FIGURE 13. THE EFFECT OF JP-4 ON RAINBOW TROUT AVERAGE LENGTH

Fish Weights

Statistical analyses of mean fish weights have not yet been completed; however, reference to Figure 14 will indicate that the data show trends similar to those of fish length. The average weight of fish exposed to 1.8 mg/ℓ WSF of JP-4 appears to be significantly lower than the other average weights during the latter stages (Day 84 – Day 112) of the experiments. It is also possible that significant weight reductions in fish exposed to lower WSF

of JP-4 concentrations may exist (Figure 14); however, statistical testing of the data will be required to verify this.

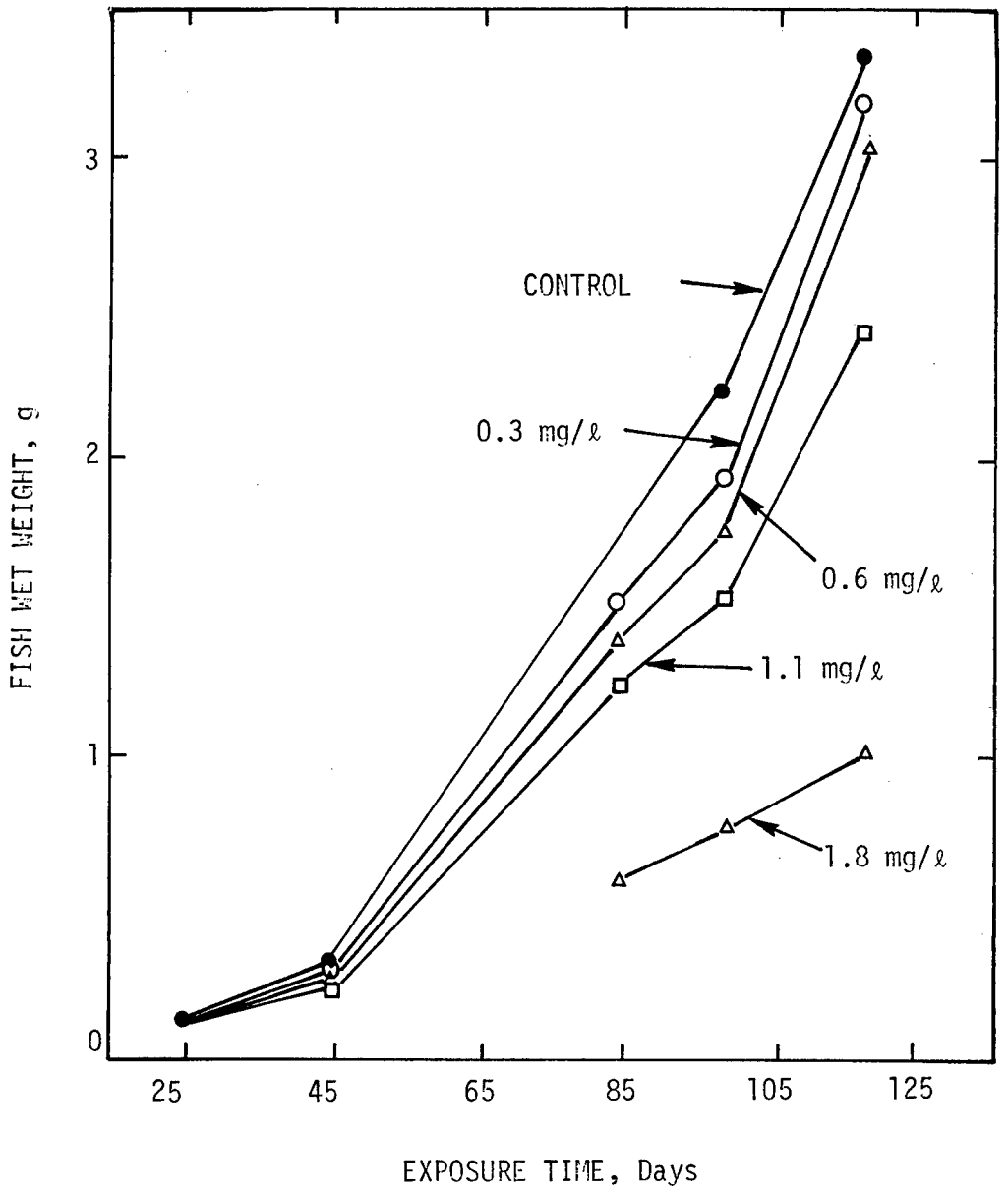


FIGURE 14. THE EFFECT OF JP-4 ON RAINBOW TROUT WEIGHT (A + B BANKS)

CONTINUOUS FLOW BIOASSAY OF HYDRAZINE

A previous 14-day (336-hr) continuous-flow hydrazine experiment (Third Annual Report AMRL-TR-78-65) indicated that at hydrazine levels of 0.53 and 0.98 mg/ℓ aufwuchs response in terms of photosynthetic index (wt.) recovered between measurements made on the 7th and 14th days of exposure. These results were inconclusive because aufwuchs development was at the detection limits of the analytical methods. Because of these factors, a 21-day exposure period experiment was conducted. Additionally, the exposure tanks were aerated more vigorously than previously in an attempt to improve aufwuchs growth. One analog tank was aerated as in the previous experiment to evaluate the effect of increased current on aufwuchs growth.

PROCEDURE

An aufwuchs growth rack containing 24 substrates was placed in each of the 9 analog tanks on Day 0. Samples for biomass, chlorophyll, and productivity analyses were taken on Days 14 and 21. Samples for measurement of aufwuchs growth were taken only on Day 14.

The selected duplicate hydrazine (active ingredient) concentrations were the following: 0 (control), 0.032, 0.18, and 0.56 mg/ℓ. On the basis of a volatility loss of 28% experienced in the previous experiment, the exposure tanks were dosed with hydrazine stock solutions some 35% stronger than required to give the desired hydrazine levels. The added strength over the stock solutions of the previous experiment was to compensate for the additional aeration. Alkalinity, chloride, and total dissolved solids were analyzed on Days 7, 14, and 21. Salinity and temperature were measured daily. Hydrazine, NH_3—N, pH, and dissolved oxygen were measured at Day 0 and thereafter every 4 days.

ANALYTICAL RESULTS

Bay water chemical characteristics are presented in Table 11. Salinity and especially temperature were higher than in previous experiments.

TABLE 11

PHYSICAL AND CHEMICAL CHARACTERISTICS OF BAY WATER

(Hydrazine Bioassay)

Constituent	Concentration ±s
Salinity, 0/00	26.1 ± 0.8
Temperature, °C	20.2 ± 2.1
DO, mg/ℓ	8.8 ± 0.5
Alkalinity, mg as $CaCO_3$/ℓ	119.0 ± 8.5
Chloride, g/ℓ	15.4 ± 0.4

Hydrazine, ammonia nitrogen, and pH data are presented in Table 12. The measured hydrazine concentrations conformed closely to the selected concentrations except for the lowest exposure which was about twice the selected value, perhaps due to difficulty in maintaining steady-state concentrations at very low levels.

Ammonia-nitrogen concentrations were erratic, and did not show any relationship to hydrazine levels or duration of the experiment. The pH of all exposure tanks was the same.

AUFWUCHS RESULTS

The results of the biomass, chlorophyll, and productivity determinations are tabulated in Table 13. Average values for each aufwuchs parameter were computed for each pair of duplicates and plotted against time in Figures 15 through 19. Figure 15 shows that all aufwuchs increased in total biomass to some degree during the 21-day experimental period. Stimulation of growth as assessed by both increases in dry matter and organic fraction over the control was caused by 0.065 mg/ℓ hydrazine; 0.17 mg/ℓ and 0.52 mg/ℓ hydrazine produced progressively greater depressions of aufwuchs growth with similar organic content to the controls. No evidence of a recovery in the growth response of the aufwuchs was noted (as had been suspected in previous experiments). These experiments indicate that the no-effect level for hydrazine on aufwuchs growth is between 0.17 and 0.52 mg/ℓ. There was very little, if any, difference in aufwuchs growth that could be ascribed to increased aeration rates (Table 13). Chlorophyll a, b, and c contents of the aufwuchs organic matter (Figure 16) were higher than the control in aufwuchs exposed to 0.17 mg/ℓ hydrazine throughout the experiment; aufwuchs exposed to 0.065 mg/ℓ and 0.52 mg/ℓ hydrazine had similar chlorophyll levels to the control aufwuchs.

The gross photosynthesis of the aufwuchs (mg O_2/aufwuchs substrate-hr) was stimulated by the presence of 0.065 mg/ℓ hydrazine, unaffected by 0.17 mg/ℓ hydrazine and depressed by 0.52 mg/ℓ hydrazine (Figure 17). Because of the changes in dry matter and chlorophyll content of aufwuchs caused by exposure to hydrazine the calculation of photosynthetic index (PI) on a dry weight and chlorophyll a basis presents a somewhat confusing pattern (Figures 18 and 19). The only clear effects are higher $PI_{(wt)}$ and $PI_{(Chl\ a)}$ values in the presence of 0.52 mg/ℓ hydrazine during the first 14 days of exposure followed by a more rapid decline than the control in $PI_{(wt)}$ and $PI_{(Chl\ a)}$ in the 0.52 mg/ℓ and possibly in the 0.17 mg/ℓ hydrazine exposed aufwuchs. From an immediate environmental point of view, gross photosynthesis would reflect best the effect of a discharge on oxygen resources. Thus, on this basis and considering the previously-discussed results on dry matter content of aufwuchs, it can be concluded that 0.065 mg/ℓ hydrazine appears to stimulate benthic biomass and photosynthetic production of oxygen; 0.17 mg/ℓ hydrazine has no effect and 0.52 mg/ℓ hydrazine represses both growth and photosynthetic oxygen production.

TABLE 12

HYDRAZINE, AMMONIA NITROGEN, AND pH CONCENTRATIONS IN HYDRAZINE BIOASSAY

Analog Tank	Hydrazine*, mg/ℓ ± s			NH_3–N*, µg/ℓ ± s		pH*
	Selected	Measured	Average	Measured	Average	
G	0.0	0.0	0.0	33.3 ± 25.8	22.2 ± 22.4	8.3 ± 0.2
H	0.0	0.0		11.1 ± 12.2		8.4 ± 0.2
C	0.032	0.075 ± 0.045	0.065 ± 0.039	11.1 ± 5.9	9.8 ± 7.0	8.3 ± 0.2
D	0.032	0.055 ± 0.055		8.5 ± 8.3		8.3 ± 0.1
A	0.18	0.19 ± 0.08	0.17 ± 0.07	23.5 ± 5.8	18.5 ± 7.1	8.3 ± 0.2
B	0.18	0.16 ± 0.06		13.5 ± 4.2		8.3 ± 0.2
K	0.56	0.51 ± 0.26	0.52 ± 0.20	15.6 ± 3.5	15.4 ± 4.1	8.3 ± 0.2
L	0.56	0.53 ± 0.15		15.1 ± 5.0		8.3 ± 0.2

* n = 6

TABLE 13

STANDING CROP AND PRODUCTIVITY OF AUFWUCHS COMMUNITIES EXPOSED TO FOUR CONCENTRATIONS OF HYDRAZINE FOR PERIODS OF 14 AND 21 DAYS

Conc. mg/L	Standing Crop: Biomass: Dry Wt. mg/Auf-Subst.		Biomass: % Organic		Chlorophyll: Chlorophyll a mg/g Dry Wt.		Chlorophyll b mg/g Dry Wt.		Chlorophyll c mg/g Dry Wt.		Pheophytin a mg/g Dry Wt.		Gross Photosynthesis mg O_2/Auf-hr		$PI_{(wt)}$ mg O_2/g Dry Wt-hr		$PI_{(chl)}$ mg O_2/mg Chl a-hr	
day→	14	21	14	21	14	21	14	21	14	21	14	21	14	21	14	21	14	21
0	13	23	15	18	2.2	2.0	0.23	0.13	0.92	0.74	0.39	0	0.71	0.86	55	37	24	19
0	16	32	15	23	1.6	2.5	0.13	0.13	0.75	1.0	0.56	0	0.75	1.6	47	51	29	20
0.075	37	69	25	34	4.8	3.8	0.22	0.23	1.6	1.6	0.11	0	2.5	3.7	67	54	14	14
0.055	37	66	23	28	4.8	3.9	0.24	0.24	1.6	1.7	0.14	0	2.6	3.8	70	58	15	15
0.19	7.0	12	17	21	5.0	5.3	0.43	0.17	2.3	1.7	0.57	0	0.58	0.77	83	64	17	12
0.16	7.0	16	16	21	4.7	7.3	0.29	0.44	1.7	2.6	0	0.13	0.76	1.2	110	76	23	10
0.51	2.0	3.0	12	19	3.5	2.0	0.10	0.07	1.0	0.33	0	0	0.30	0.19	150	63	43	32
0.53	2.0	3.0	12	17	3.0	2.3	0.25	0.03	1.0	0.67	0	0	0.17	0.050	85	17	28	7.0
0*	12		15		1.4		0.16		0.75									

* Aeration comparison sample.

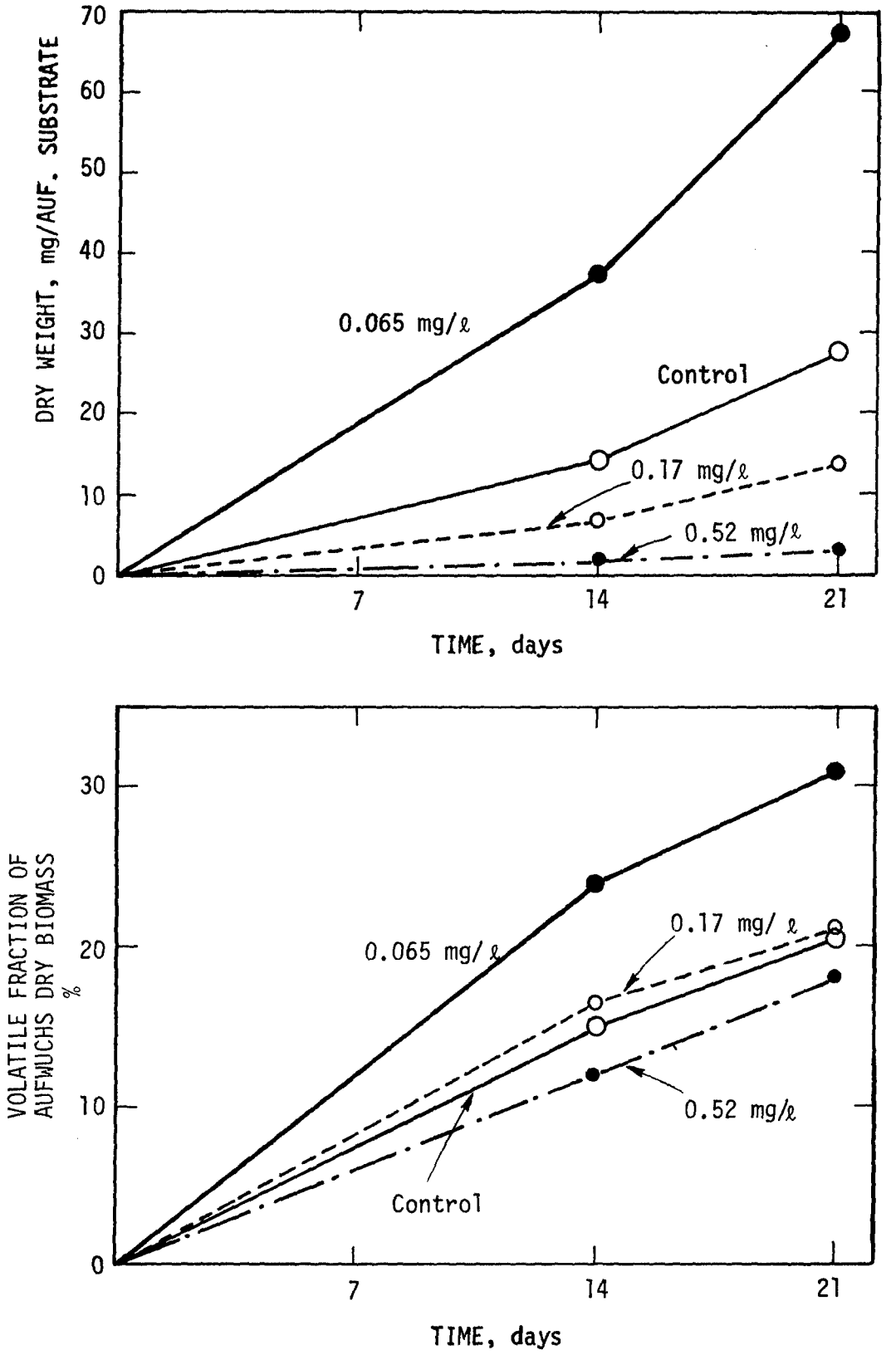


FIGURE 15. EFFECT OF HYDRAZINE ON AUFWUCHS DRY WEIGHT AND ORGANIC MATTER CONTENT (HYDRAZINE BIOASSAY)

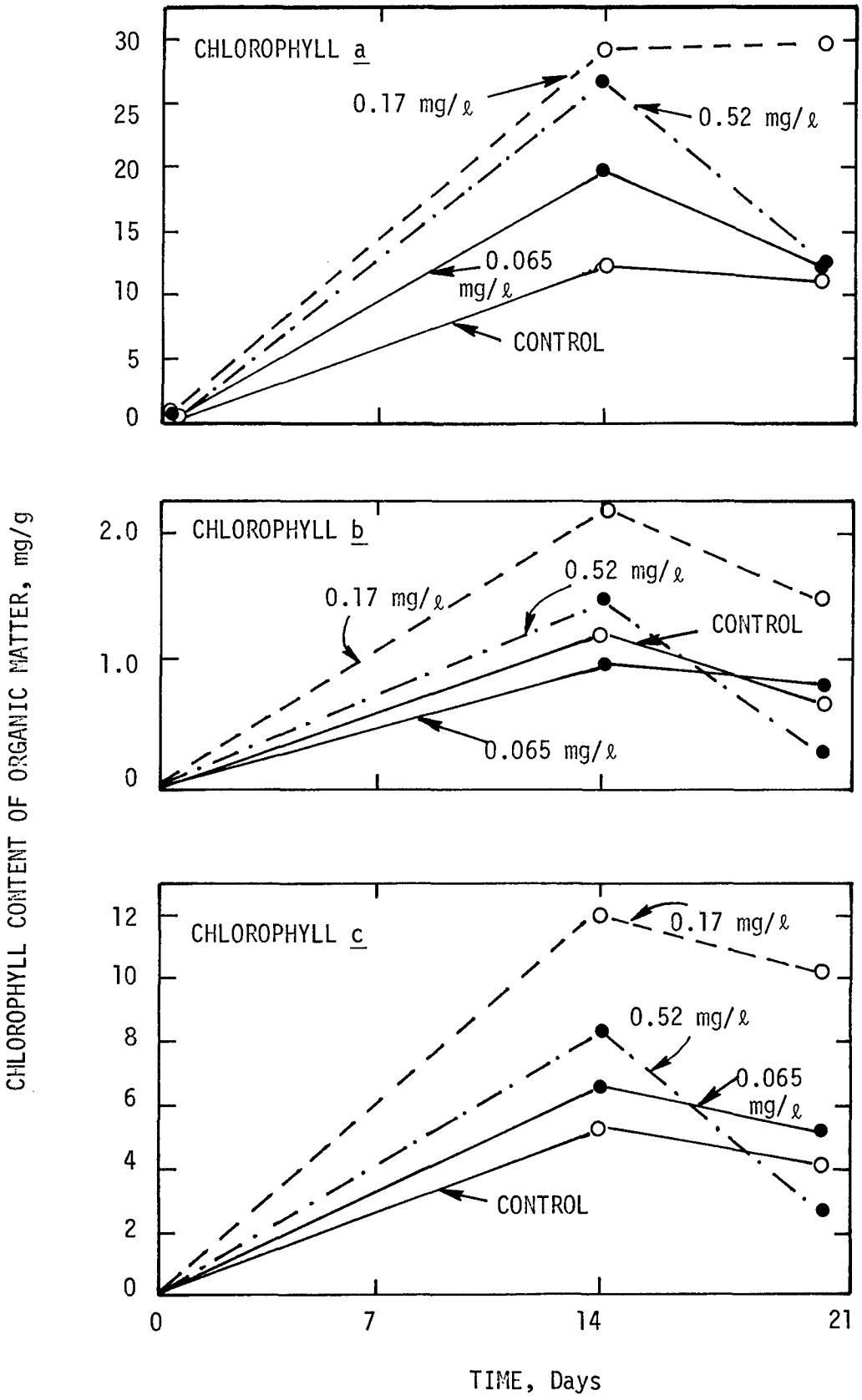


FIGURE 16. CHLOROPHYLL a, b, AND c CONTENT OF AUFWUCHS ORGANIC MATTER (HYDRAZINE BIOASSAY)

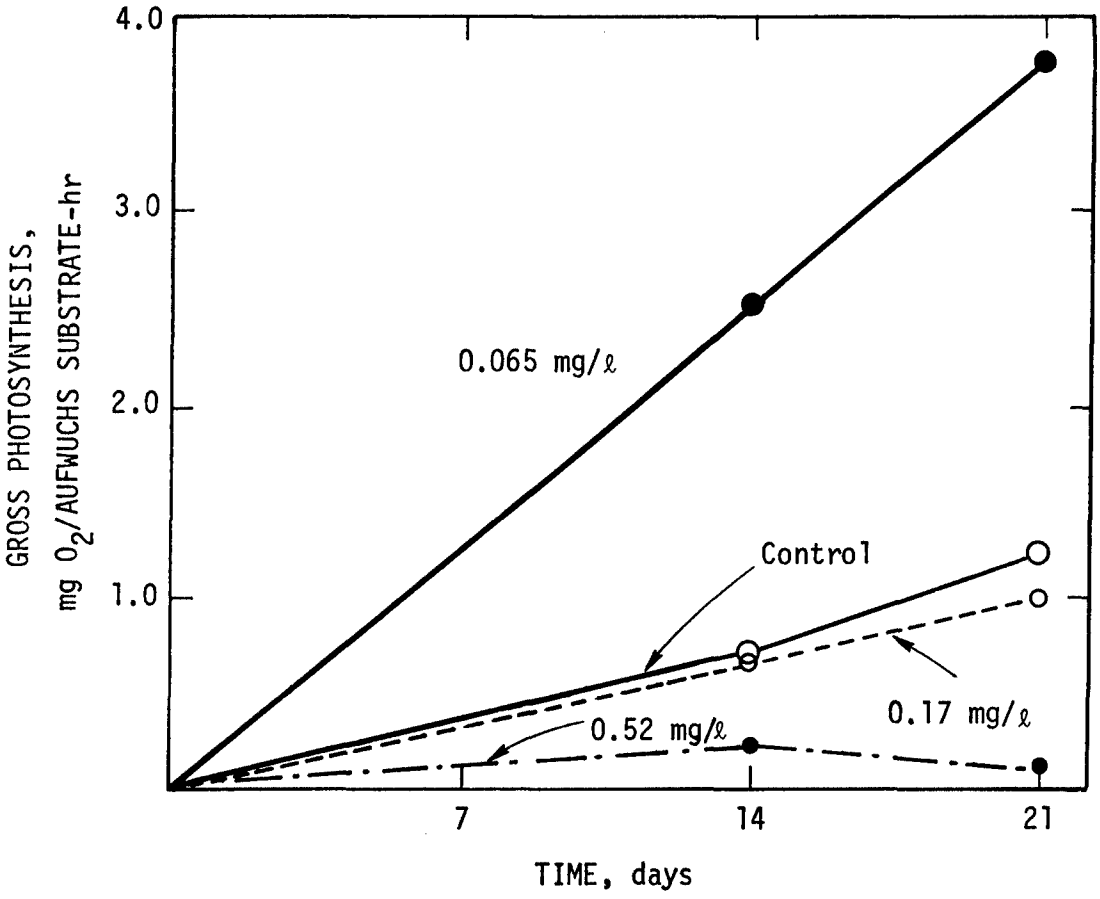


FIGURE 17. GROSS PHOTOSYNTHESIS OF AUFWUCHS (HYDRAZINE BIOASSAY)

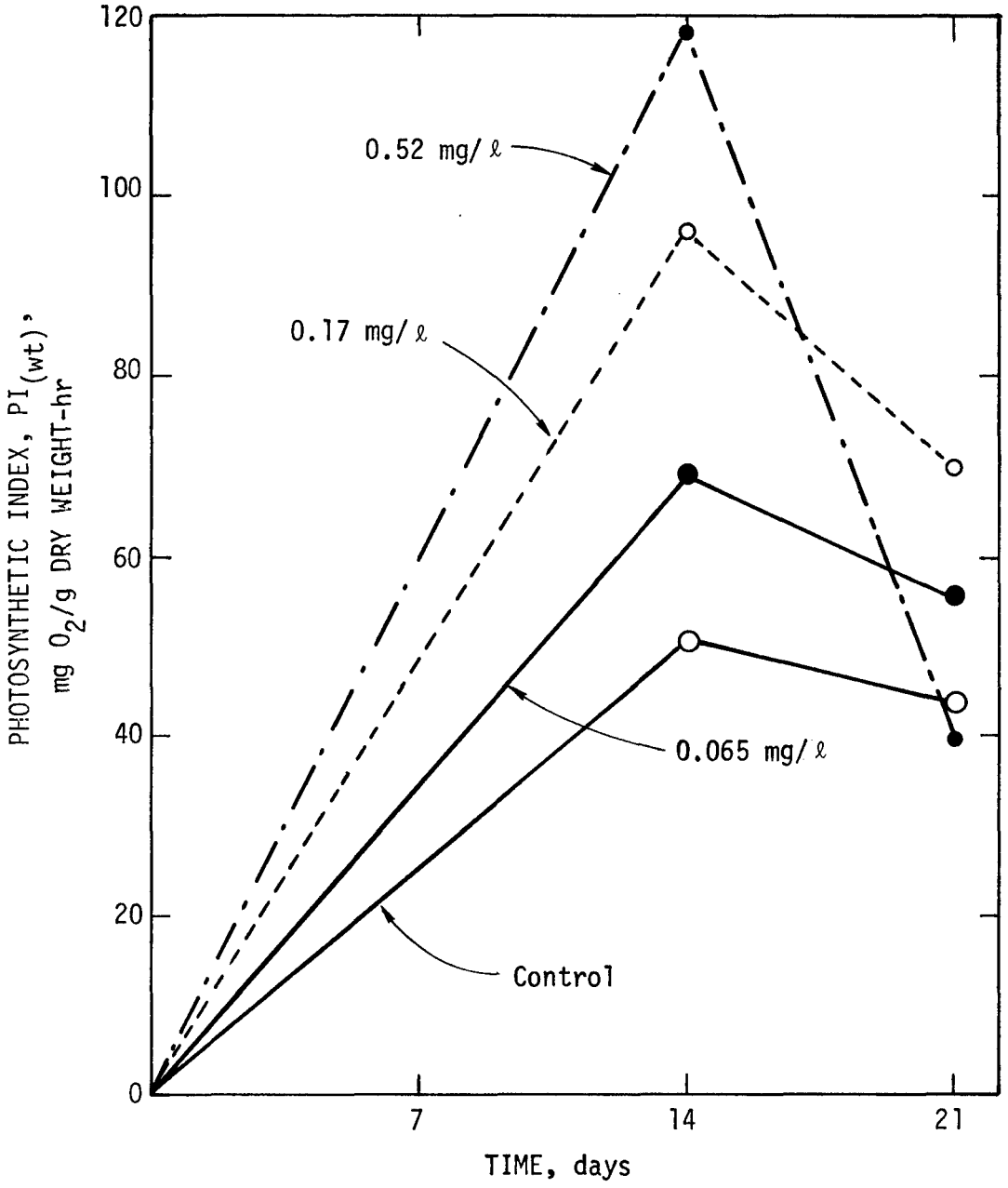


FIGURE 18. PHOTOSYNTHETIC INDEX$_{(wt)}$ OF AUFWUCHS EXPOSED TO HYDRAZINE

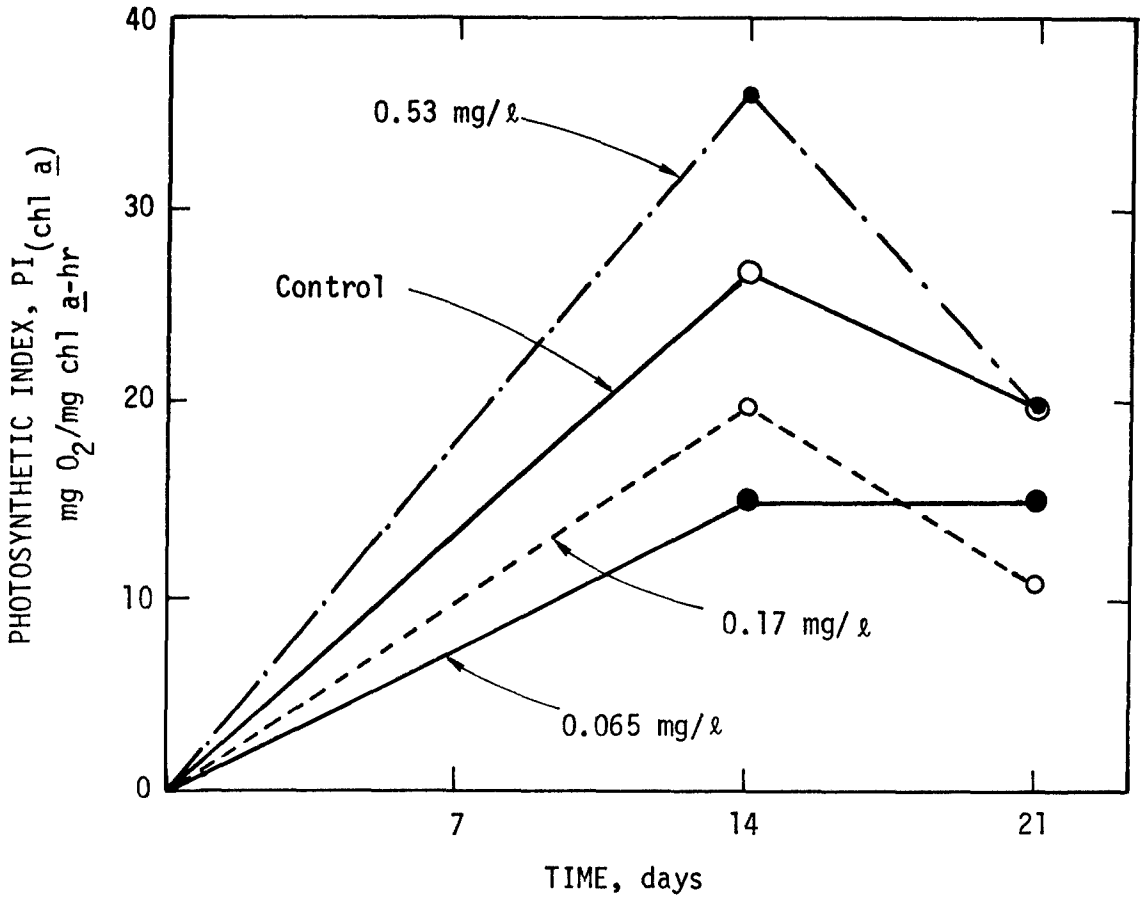


FIGURE 19. PHOTOSYNTHETIC INDEX$_{(\text{chl } a)}$ OF AUFWUCHS EXPOSED TO HYDRAZINE

CONTINUOUS FLOW BIOASSAYS OF MMH

FOURTEEN-DAY SUMMER BIOASSAY

Stickleback (Gasterosterus aculeatus), crabs (Hemigrapsus nudus), mussels (Mytilus edulis), and aufwuchs were exposed to MMH in a 14-day (336-hr) continuous-flow saline water bioassay to determine LC50 values and the effect on aufwuchs growth and activity.

Procedure

Thirty stickleback (36.1 ± 2.7 mm length), 10 crabs (23.8 ± 3.5 mm (carapace width)), and 10 mussels (33.4 ± 3.6 mm longest dimension) were added to each analog tank and segregated by fish cages and Nytex bags. All test organisms were acclimated to Bay water (salinity, 30.6 ± 0.4 0/00; temperature, 18.8 ± 1.0°C; pH, 8.04 ± 0.02) for one week prior to the start of the experiment. At the start of the bioassay an aufwuchs growth rack containing 24 substrates was placed in each of the 8 analog tanks. Aufwuchs

samples were taken after 168 and 336 hours' exposure to MMH to determine biomass, chlorophyll concentration, and photosynthetic activity.

MMH concentrations were 0, 0.01, 0.1, and 1.0 mg/ℓ. Stock dosing solutions were made up with a 60% greater concentration than needed to produce these levels to account for decay of MMH (determined from a preliminary experiment).

Alkalinity, chloride, and total dissolved solids were analyzed at 0 hr, 168 hr, and 336 hr; salinity and temperature were measured daily; MMH, NH_3–N, pH, and DO were measured at 0 hr and every 4 days thereafter.

Results

Bay water chemical characteristics are presented in Table 14; salinity appears to be higher than in previous experiments. Measured MMH concentrations were considerably less than desired levels (Table 15). There was a significant (99% confidence level) difference in NH_3–N values in the highest MMH exposure tanks (~47 μg/ℓ NH_3–N) and the other tanks (20-25 μg/ℓ NH_3–N), possibly because of the formation of breakdown products of MMH which contributed toward the NH_3–N level in the water. The pH of all the exposure tanks was identical (Table 15).

TABLE 14

PHYSICAL AND CHEMICAL CHARACTERISTICS OF BAY WATER
(MMH BIOASSAY)

Constituent	Concentration
Salinity, 0/00	30.7 ± 0.7
Temperature, °C	19.1 ± 1.6
DO, mg/ℓ	8.1 ± 0.5
Alkalinity, mg/ℓ	127 ± 2.5
Chloride, g/ℓ	16.3 ± 0.2

Stickleback and crab and mussel mortality data are presented in Tables 16, 17, and 18. Stickleback were the most sensitive of the 3 test organisms. Although 100% mortalities were observed in the crab and mussel, these responses occurred at later exposure times than those exhibited by the stickleback. Furthermore, no mortalities of the crabs and mussels occurred at MMH concentrations of 0.004 mg/ℓ and 0.012 mg/ℓ.

While the estimated 336-hr LC50 for stickleback continuously exposed to MMH is 0.011 mg/ℓ (a value consistent with the static 96-hr LC50 of 0.36 mg/ℓ reported in the Third Annual Report AMRL-TR-78-65, it should be noted that there was considerable mortality (17%) in the control. The exact cause for this is not known; the fish used appeared healthy during acclimation; however, the problem may have been associated with the high silt content of the Bay water at the time of the experiment. Because of this, the above LC50 value must be regarded as tentative. However, it is quite clear that MMH is far more toxic than hydrazine (336-hr LC50 – 1.1 mg/ℓ). Also, in contrast to the

TABLE 15

MMH, AMMONIA NITROGEN, AND pH LEVELS IN MMH BIOASSAY
SUMMER STUDY

Analog Tank	Monomethyl Hydrazine*, mg/ℓ			NH_3-N*, µg/ℓ		pH*
	Selected	Measured	Average	Measured	Average	Measured
C	0.0	0.0	0.0	30.7 ±19.5	24.4 ±18.2	8.1 ±0.2
D	0.0	0.0		18.0 ±16.2		8.2 ±0.2
I	0.01	0.004±0.	0.005± 0.005	24.2 ±15.0	22.9 ±14.5	8.1 ±0.2
J	0.01	0.005±0.006		21. ±15.6		8.1 ±0.2
K	0.10	0.013±0.010	0.012± 0.010	20.3 ±15.7	20.4 ±14.1	8.1 ±0.2
L	0.10	0.012±0.010		20.5 ±14.1		8.0 ±0.2
E	1.0	0.15 ±0.091	0.149± 0.092	47.4 ± 7.3	46.9 ±5.4	8.0 ±0.1
F	1.0	0.15 ±0.10		46.4 ±3.4		8.0 ±0.04

* n = 5

TABLE 16

MORTALITY OF STICKLEBACK EXPOSED TO MMH IN CONTINUOUS FLOW BIOASSAY

Analog Tank	MMH Conc. mg/L	Number of Test Organisms	Cumulative Number of Test Organisms Dead After Hour:														Mortality at 336-hr %
			24	48	72	96	120	144	168	192	216	240	264	288	312	336	
C	0	30	1	1	2	2	2	2	2	3	4	4	4	5	5	5	17
D	0	30	0	0	2	2	2	4	4	5	5	5	5	5	5	5	17
I	0.004	30	0	0	1	1	3	4	7	7	7	7	7	7	7	7	23
J	0.005	30	2	2	2	2	3	3	3	3	3	3	3	3	3	4	13
K	0.013	30	0	0	0	2	8	13	14	14	15	15	15	15	15	15	50
L	0.012	30	0	0	0	2	8	14	15	15	15	15	15	15	15	15	60
E	0.15	30	1	30	30	30	30	30	30	30	30	30	30	30	30	30	100
F	0.15	30	2	30	30	30	30	30	30	30	30	30	30	30	30	30	100

TABLE 17

MORTALITY OF CRAB (Hemigrapsus nudus) EXPOSED TO MMH IN CONTINUOUS FLOW BIOASSAY

Analog Tank	MMH Conc. mg/L	Number of Test Organisms	Cumulative Number of Test Organisms Dead After Hour:													Mortality at 336-hr %
			24	48	72	96	120	144	168	192	216	240	264	288	312	336	
C	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I	0.004	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J	0.005	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K	0.013	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	0.012	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0.15	10	0	2	5	10	10	10	10	10	10	10	10	10	10	10	100
F	0.15	10	0	2	5	9	10	10	10	10	10	10	10	10	10	10	100
F	0.15	10*	0	8	10	10	10	10	10	10	10	10	10	10	10	10	100

* Hemigrapsus oregonensis

TABLE 18

MORTALITY OF MUSSEL EXPOSED TO MMH IN CONTINUOUS FLOW BIOASSAY

Analog Tank	MMH Conc. mg/L	Number of Test Organisms	Cumulative Number of Test Organisms Dead After Hour:													Mortality at 336-hr %
			24	48	72	96	120	144	168	192	216	240	264	288	312	336	
C	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I	0.004	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J	0.005	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K	0.013	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	0.012	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0.15	10	0	0	0	0	0	2	7	10	10	10	10	10	10	10	100
F	0.15	10	0	0	0	0	0	9	9	10	10	10	10	10	10	10	100

hydrazine experiments, MMH did not appear to have caused stressed behavior in stickleback such as disorientation in swimming and increased respiration.

For both crabs and mussels it was not possible to compute an LC50 value because there was 100% mortality in the highest MMH concentration but no deaths in the two lower MMH concentrations (Tables 17 and 18). Because of this, it can only be stated that the 336-hr LC50 values for Hemigrapus nudus and Mytilus edulis lie between 0.012 and 0.15 mg/ℓ MMH. Mortality rate at 0.15 mg/ℓ MMH for the crab was more rapid for H. nudus (100% mortality in 4 days) than for M. edulis (100% mortality in 9 days). Compared to previous work with H. oregonansis (100% mortality in 3 days), the mortality rate of H. nudus was lower, possibly because of its larger carapace size (23.5 mm wide compared with 17.9 mm wide).

MMH at a concentration of 0.15 mg/ℓ had a severely toxic effect on aufwuchs (Table 19 and Figures 20 through 22) as assessed by all responses measured at 336 hr (aufwuchs dry weight, aufwuchs volatile matter fraction, gross photosynthesis, chlorophyll a, b, and c levels and photosynthetic indices). MMH levels of 0.005 mg/ℓ and 0.012 mg/ℓ did not appear to adversely affect the gross photosynthesis or the photosynthetic index of aufwuchs during the 336-hr exposure period. These concentrations of MMH reduced both measures of aufwuchs growth (dry weight and % volatile matter) at 336 hr of exposure but not after 168 hr of exposure. Aufwuchs dry weight at 336-hr exposure was approximately 50% of control values; the % volatile matter was less severely affected (Figure 20).

It is rather difficult to conclude the no-effect level for MMH on aufwuchs from these experiments. For photosynthetic activity it appears to be between 0.012–0.15 mg/ℓ; for growth the level appears lower, between 0–0.012 mg/ℓ. On these bases, MMH appears to be much more toxic to aufwuchs than hydrazine (no-effect level 0.17–0.52 mg/ℓ).

FOURTEEN-DAY WINTER STUDY

Introduction

Because of the high control mortality encountered in the previously reported study, and also because of the abnormally high silt content of the Bay water in that study, a continuous flow bioassay of MMH on stickleback was repeated under "winter" conditions.

Procedure

Sticklebacks (10 per tank, mean fish length 39.8 mm ± 1.4 mm) were acclimated for 8 weeks in 8 analog tanks.

Water Quality Results

Water quality during acclimation and bioassay periods is shown in Table 20; mean hydraulic residence time in the analog tanks was 17.4 hr. Salinity was marginally low and temperature was significantly lower than in the previous summer MMH bioassay. As in the previous bioassay, alkalinity and chloride concentration were measured at the start, midpoint, and end of

TABLE 19

STANDING CROP AND PRODUCTIVITY OF AUFWUCHS COMMUNITIES EXPOSED TO FOUR CONCENTRATIONS OF MMH FOR PERIODS OF 168 AND 336 HOURS

MMH Conc. mg/L	Standing Crop: Biomass: Dry Wt. mg/Auf.		Biomass: Dry Wt. % Organic		Chlorophyll: Chlorophyll a mg/g Dry Wt.		Chlorophyll: Chlorophyll b mg/g Dry Wt.		Chlorophyll: Chlorophyll c mg/g Dry Wt.		Chlorophyll: Pheophytin a mg/g Dry Wt.		Gross Photosynthesis mg O_2/Auf-hr		$PI_{(wt)}$ mg O_2/g Dry Wt-hr		$PI_{(chl)}$ mg O_2/mg Chl a-hr	
hr →	168	336	168	336	168	336	168	336	168	336	168	336	168	336	168	336	168	336
0	28	51	10	10	9.4	4.0	0.57	0.27	4.1	1.7	0	0.65	2.2	1.2	80	23	8.6	5.7
0	25	54	9	11	10	3.8	0.56	0.33	4.3	1.8	0	0.35	2.6	1.3	100	24	10	6.2
0.004	23	31	8	8	12	5.9	0.78	0.42	5.5	2.5	0	0.10	2.6	0.90	110	29	9.5	4.9
0.005	25	24	9	7	8.6	7.4	0.56	0.54	3.8	3.0	0	0.17	2.5	0.90	98	38	11	5.1
0.013	23	35	8	10	10	6.1	0.65	0.46	4.5	2.7	0	0.17	2.4	0.97	100	28	10	4.6
0.012	23	22	8	8	10	9.6	0.65	0.68	4.5	4.1	0	0.09	2.1	0.92	93	42	9.3	4.4
0.15	1.0	0.40	7	2	1.0	10	0	2.50	1.0	0	1.0	0	0.15	-0.13	150	-320	150	-33
0.15	2.0	2.0	8	3	0.50	2.5	0	0.50	0	0.50	0	1.0	0.14	-0.06	70	- 30	140	-30

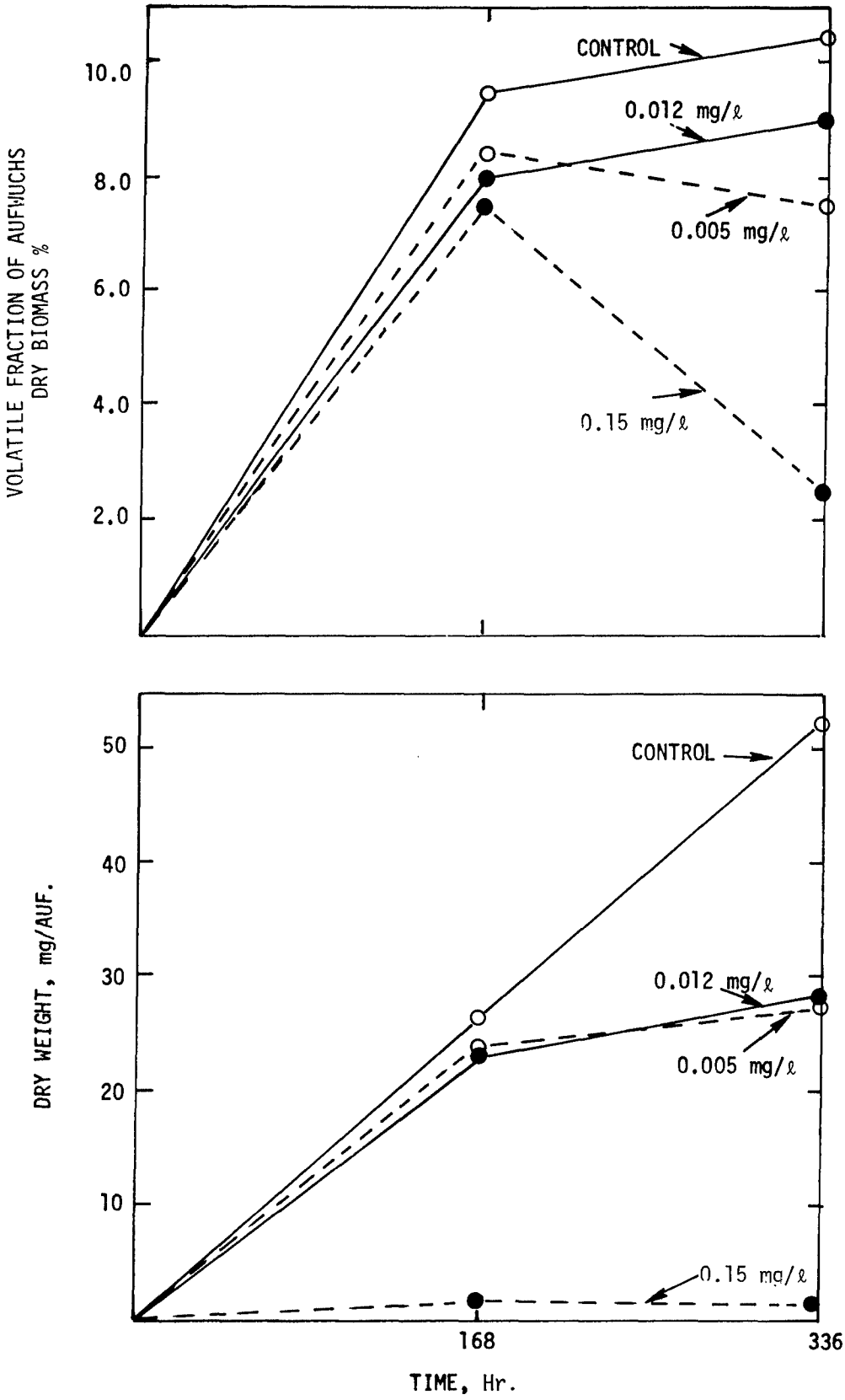


FIGURE 20 EFFECT OF MMH ON AUFWUCHS DRY WEIGHT AND ORGANIC MATTER CONTENT (MMH BIOASSAY)

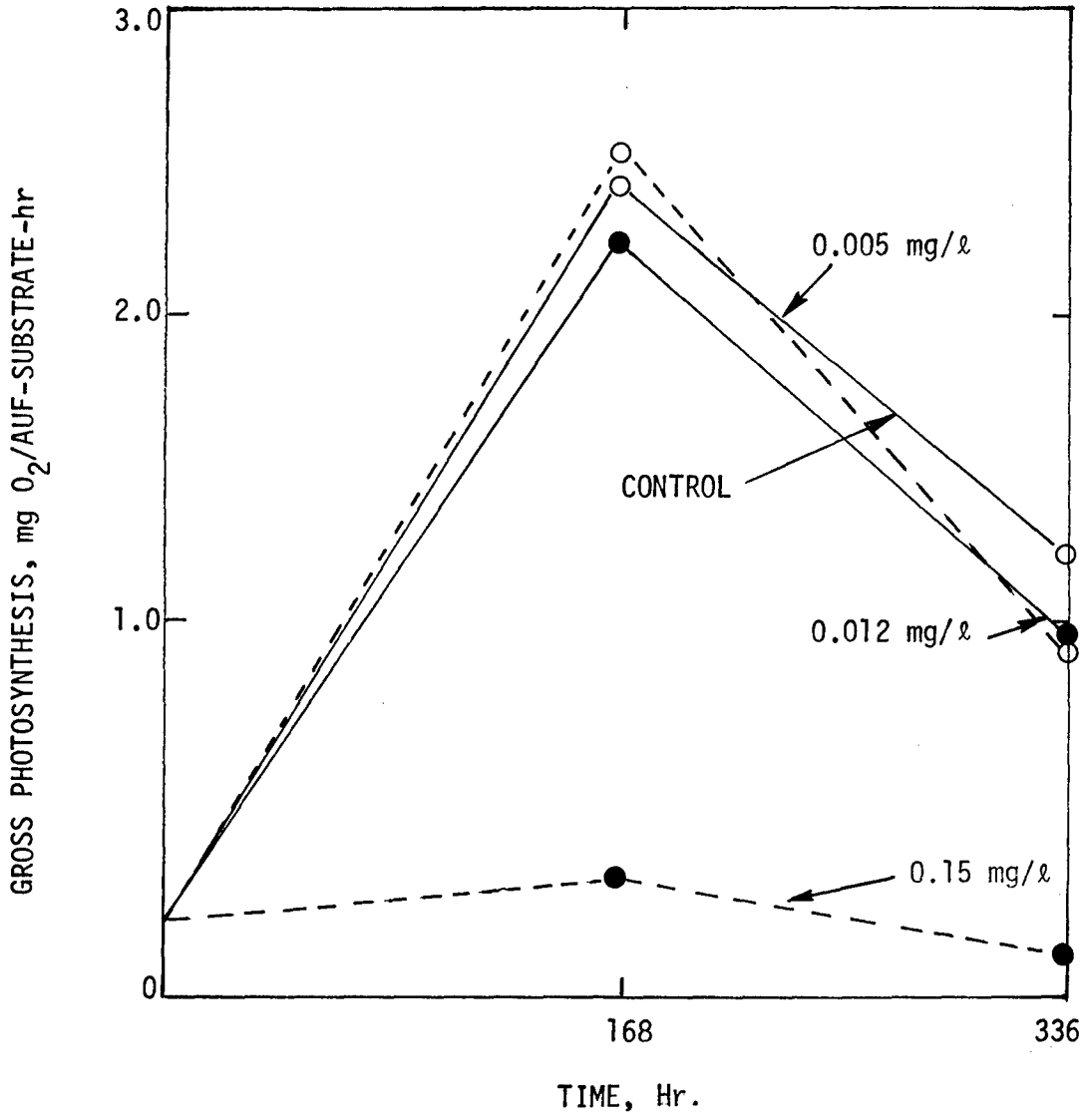


FIGURE 21. GROSS PHOTOSYNTHESIS OF AUFWUCHS (MMH BIOASSAY)

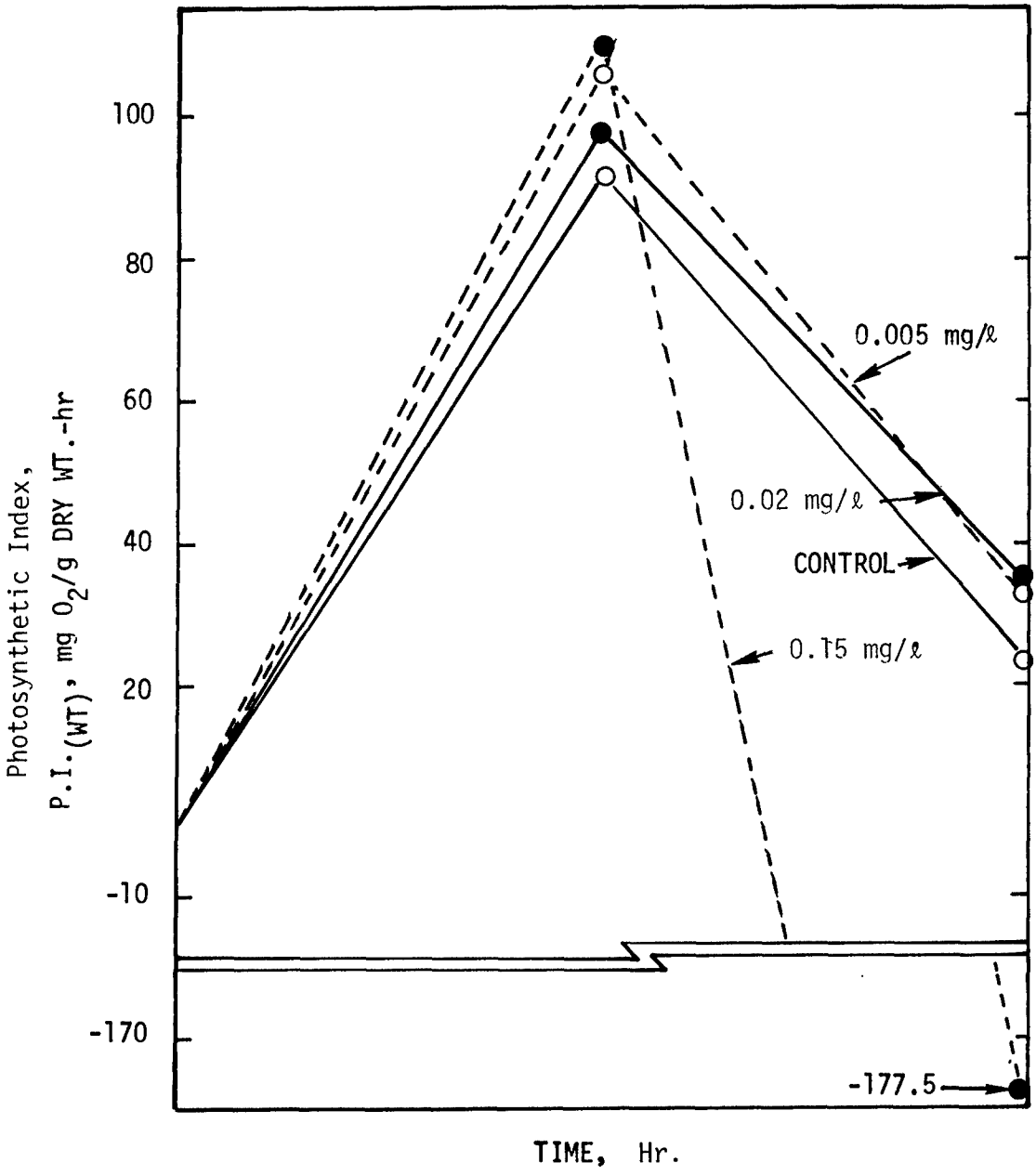


FIGURE 22. PHOTOSYNTHETIC INDEX$_{(wt)}$ OF AUFWUCHS EXPOSED TO MMH

TABLE 20

PHYSICAL AND CHEMICAL CHARACTERISTICS OF BAY WATER DURING ACCLIMATION AND BIOASSAY PERIODS

Constituent	Concentration	
	Bioassay Period	Acclimation Period
Salinity 0/00	29.6 ± 0.85	27 ± 2.3
Temperature, °C	8.3 ± 1.28	11.6 ± 2.8
DO, mg/ℓ	10.0 ± 0.4	
Alkalinity, mg $CaCO_3$/ℓ	118 ± 2.9	
Chloride, g/ℓ	15.1 ± 1.0	
pH	8.0	7.9 ± 0.1

the experiment. MMH, NH_3–N, pH, and DO were measured at 4-day intervals. Duplicate MMH levels of 0, 0.01, 0.1, and 0.50 mg/ℓ were aimed for; but Table 21 shows that even though feed MMH levels were elevated to account for MMH losses, the target MMH levels were not achieved. No significant variation in NH_3–N levels or in pH was discernible in the various exposure tanks (Table 21).

Mortality Results

Mortality results presented in Table 22 show that no deaths occurred in the control or at the two lower exposure levels (0.025 and 0.069 mg/ℓ MMH). However, mortality commenced at the highest exposure level (0.26 mg/ℓ MMH) by 120 hr and proceeded until there was 100% mortality by 264 hr.

These results suggest that the 336-hr LC50 for MMH is in the range 0.07 and 0.26 mg/ℓ compared with the value of 0.011 mg/ℓ obtained in the above 14-day summer study. Several factors may account for this difference – the most important of which is probably the lower temperature (8°C) in this experiment compared with 18°C in the previous summer bioassay. Significantly less fish movement was noted in the current bioassay compared with the vigorous swimming activity in the summer bioassay.

If the results of the summer bioassay are "corrected" by interpolation for the 17% mortality in the controls, they yield an LC50 value of 0.035 mg/ℓ. If we assume that the toxic effects of MMH on stickleback obey the general Arrhenius relationship for biological systems (doubling of activity for each 10°C rise in temperature) then the toxicity at 8°C should be approximately one-half that at 18°C. Since the estimated range of LC50 values at 8°C is 0.07–0.26 mg/ℓ MMH, and the interpolated LC50 at 18°C is 0.035 mg/ℓ MMH, these results provide tentative support to the idea that over the temperature range 8–18°C the acute toxicity of MMH to stickleback follows the Arrhenius relationship.

TABLE 21

MMH, AMMONIA NITROGEN, AND pH LEVELS IN MMH BIOASSAY
WINTER STUDY

Analog Tank	MMH * mg/ℓ Selected	MMH * mg/ℓ Measured	MMH * mg/ℓ Average	NH_3-N,* µg/ℓ Measured	NH_3-N,* µg/ℓ Average	pH* Measured
A	0.0	0.0	0.0	40.6 ±20.9	37.77±18.15	8.0 ±0.1
B	0.0	0.0		35.0 ±17.6		8.0 ±0.1
C	0.01	0.022±0.012	0.025±0.014	28.6 ±12.6	26.42±12.20	8.0 ±0.1
D	0.01	0.029±0.017		24.2 ±13.2		8.0 ±0.1
K	0.10	0.062±0.026	0.069±0.026	35.4 ±20.5	32.88±16.67	8.0 ±0.1
L	0.10	0.076±0.028		30.3 ±14.4		8.0 ±0.1
I**	0.56	0.25 ±0.078	0.26 ±0.076	34.2 ±11.8	30.33±8.94	8.0 ±0.04
J**	0.56	0.27 ±0.089		26.4 ±3.8		8.0 ±0.1

* n = 4
** n = 3

TABLE 22

MORTALITY OF STICKLEBACK EXPOSED TO MMH IN CONTINUOUS FLOW BIOASSAY

Analog Tank	MMH Conc. mg/ℓ	Number of Test Organisms	Cumulative Number of Test Organisms Dead After Hour:														Mortality at 336-hr %
			24	48	72	96	120	144	168	192	216	240	264	288	312	336	
A	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C	0.022	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	0.029	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K	0.062	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	0.076	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I	0.25	10	0	0	0	0	5	7	8	9	10	10	10	10	10	10	100
J	0.27	10	0	0	0	0	1	6	9	9	9	9	10	10	10	10	100

CONTINUOUS FLOW BIOASSAYS OF UDMH

FOURTEEN-DAY SUMMER BIOASSAY

Introduction

A 14-day continuous flow bioassay was conducted to determine the toxicity of UDMH to stickleback, the crab (Hemigrapsus nudus), mussel (Mytilus edulis), and aufwuchs.

Procedure

Thirty stickleback (37.2 ± 2.7 mm long), 10 crabs (25.5 ±4.6 mm carapace width), and 10 mussels (33.2 ± 5.1 mm long) were placed in each analog tank and acclimated to Bay water at a flow of 4 ℓ/min (residence time of 17.4 hr) for one week prior to the start of the experiment.

Twenty-four aufwuchs substrates were placed in each analog tank at the start of the experiment. Aufwuchs were sampled after 168 and 336 hr exposure for biomass, chlorophyll, and photosynthetic response.

UDMH stock solutions were metered into the analog tanks for a 24-hour period of equilibration before initiating the experiment. Alkalinity and chloride were analyzed at 0 , 168 , and 336 hr; salinity and temperature were measured daily. UDMH, NH_3–N, pH, and DO were measured at 0 hr and every 72 hr thereafter.

Results

The Bay water characteristics during acclimation and experimental periods presented in Table 23 show that water quality was similar to that in the 336-hr summer MMH experiment except that temperature was slightly higher.

TABLE 23

PHYSICAL AND CHEMICAL CHARACTERISTICS OF BAY WATER

	Concentrations	
Constituent	During Bioassay	During Acclimation Period
Salinity, 0/00	30.4 ± 0.9	30.8 ± 0.4
Temperature, C	20.4 ± 1.2	22.2 ± 0.2
DO, mg/ℓ	7.9 ± 0.4	
Alkalinity, mg/ℓ	122 ± 2.0	
Chloride, g/ℓ	15.9 ± 0.2	
pH		8.0 ± 0.02

Results of UDMH, NH_3–N, and pH analyses, presented in Table 24, show that the average measured UDMH concentrations were 0, 0.18, 0.46, and 1.1 mg/ℓ. The large standard deviations of UDMH concentration indicate the difficulty in measuring and maintaining low UDMH values. No difference was noted between a surface and mid-depth UDMH concentration as in a previous "spill bioassay" (Third Annual Report AMRL-TR-78-65). This indicates that the modifications of the mixing apparatus provide adequate mixing of UDMH and Bay water.

No significant differences were observed in either NH_3–N or pH measurements between exposure tanks.

Mortality Results

The two highest UDMH concentrations (0.46 and 1.1 mg/ℓ) caused 100% mortality of stickleback (between 72 and 120 hr), of crabs (after 192 hr for 1.1 mg/ℓ UDMH, after 288 hr for 0.46 mg/ℓ UDMH), and mussels (after 192 hr for 1.1 mg/ℓ UDMH, after 240 hr for 0.4 mg/ℓ UDMH). UDMH concentrations (0.18 mg/ℓ) permitted 100% survival of crab and mussel after 336 hr and an average of 48% survival of stickleback after 336 hr exposure. These data are unsuitable for calculating the 336-hr LC50 values for any of these species; an estimate of the 336-hr LC50 for stickleback is 0.2 mg/ℓ UDMH.

Aufwuchs Results

Table 25 and Figure 23 show that standing crops were reduced at UDMH levels of 0.46 and 1.1 mg/ℓ but not at 0.18 mg/ℓ after 336 hr of exposure. No clearly discernible effect on chlorophyll a, b, and c and pheophytin a levels could be ascribed to the presence of UDMH.

Gross photosynthesis of aufwuchs was adversely affected by UDMH at 168 hr (for 0.46 and 1.1 mg/ℓ UDMH) and 336 hr (for all UDMH levels tested) (Figure 24). However, because of the greater biomass levels in the control and the aufwuchs exposed to 0.18 mg/ℓ UDMH, photosynthetic indices were not consistently different at 168 hr and 336 hr of exposure (Table 25).

TWENTY-ONE DAY WINTER BIOASSAY

Introduction

Because the previous continuous flow bioassay on UDMH had not produced sufficient data to determine a 336 hr LC50 value for stickleback, a second bioassay was conducted at lower UDMH levels. The second bioassay was conducted at a lower temperature (11.8°C) than the first bioassay (20.4°C).

Procedure

Twenty stickleback (average length, 38.6 ± 1.2 mm) were exposed to each of the following duplicate UDMH levels: 0, 0.044, 0.10, and 0.20 mg/ℓ, following acclimation for 12 days. Bay water flow rates of 4.0 ℓ/min gave an analog average hydraulic residence time of 17.4 hr. Alkalinity and chloride

TABLE 24

UDMH, AMMONIA NITROGEN, AND pH VALUES IN THE 336-hr CONTINUOUS FLOW BIOASSAY OF UDMH

Concentration, mean ±s

Analog Tank	UDMH* Concentration mg/ℓ			NH_3-N* Concentration, µg/ℓ		pH*
	Selected	Measured	Average	Measured	Average	Measured
A	0.0	0.0	0.0	35.9 ±39.9	20.8 ±31.6	8.1 ±0.2
B	0.0	0.0		5.7 ± 9.4		8.1 ±0.1
I	0.10	0.16±0.12	0.18±0.13	14.5 ±17.7	14.4 ±17.8	8.0 ±0.2
J	0.10	0.19±0.15		14.2 ±20.0		8.0 ±0.1
K	1.0	0.41±0.15	0.46±0.19	40.8 ±15.6	40.3 ±13.5	8.0 ±0.1
L	1.0	0.51±0.23		39.8 ±12.9		8.0 ±0.1
E	1.8	1.2 ±0.68	1.1 ±0.49	34.4 ±18.0	36.1 ± 14.0	8.0 ±0.1
F	1.8	0.96±0.17		37.7 ±10.5		8.0 ±0.1

*n = 5

TABLE 25

STANDING CROP AND PRODUCTIVITY OF AUFWUCHS COMMUNITIES EXPOSED TO UDMH FOR PERIODS OF 168 AND 336 HOURS

UDMH Conc mg/ℓ	Standing Crop: Biomass: Dry Wt. mg/Auf.		Standing Crop: Biomass: Dry Wt. % Organic		Standing Crop: Chlorophyll: Chlorophyll a mg/g Dry Wt.		Standing Crop: Chlorophyll: Chlorophyll b mg/g Dry Wt.		Standing Crop: Chlorophyll: Chlorophyll c mg/g Dry Wt.		Standing Crop: Chlorophyll: Pheophytin a mg/g Dry Wt.		Productivity: Gross Photosynthesis mg O_2/Auf-hr		Productivity: $PI_{(wt)}$ mg O_2/g Dry Wt-hr		Productivity: $PI_{(chl)}$ mg O_2/mg Chl a-hr	
hr→	168	336	168	336	168	336	168	336	168	336	168	336	168	336	168	336	168	336
0	11	32	15	12	4.1	6.6	0.46	0.57	1.8	2.8	0.04	0.40	0.47	2.9	43	92	10	14
0	16	40	13	11	7.0	4.9	0.50	0.59	2.8	2.1	0.06	0.62	1.1	2.3	70	58	10	12
0.16	14	45	11	9.7	7.5	6.1	0.44	0.57	2.6	2.5	0.02	0.88	0.83	1.5	60	32	8.1	5.3
0.19	13	37	11	10	7.2	6.2	0.39	0.62	2.6	2.7	0.09	0.44	0.75	2.0	59	54	8.2	8.7
0.41	7.9	24	11	9.5	3.7	6.9	0.63	0.62	2.0	2.9	0.49	0.75	0.55	1.34	70	55	19	8.0
0.51	6.1	23	9.3	9.1	3.9	8.5	0.98	0.73	2.8	4.0	0.82	0.35	0.34	1.7	56	71	14	8.3
1.2	6.7	12	11	10	3.7	7.8	0.30	0.69	1.5	3.3	0.48	1.1	0.16	1.0	24	88	6.4	11
1.0	7.7	14	11	8.4	3.5	7.8	0.39	0.72	1.4	3.1	0.23	0.94	0.36	0.83	47	60	13	7.8

n = 5

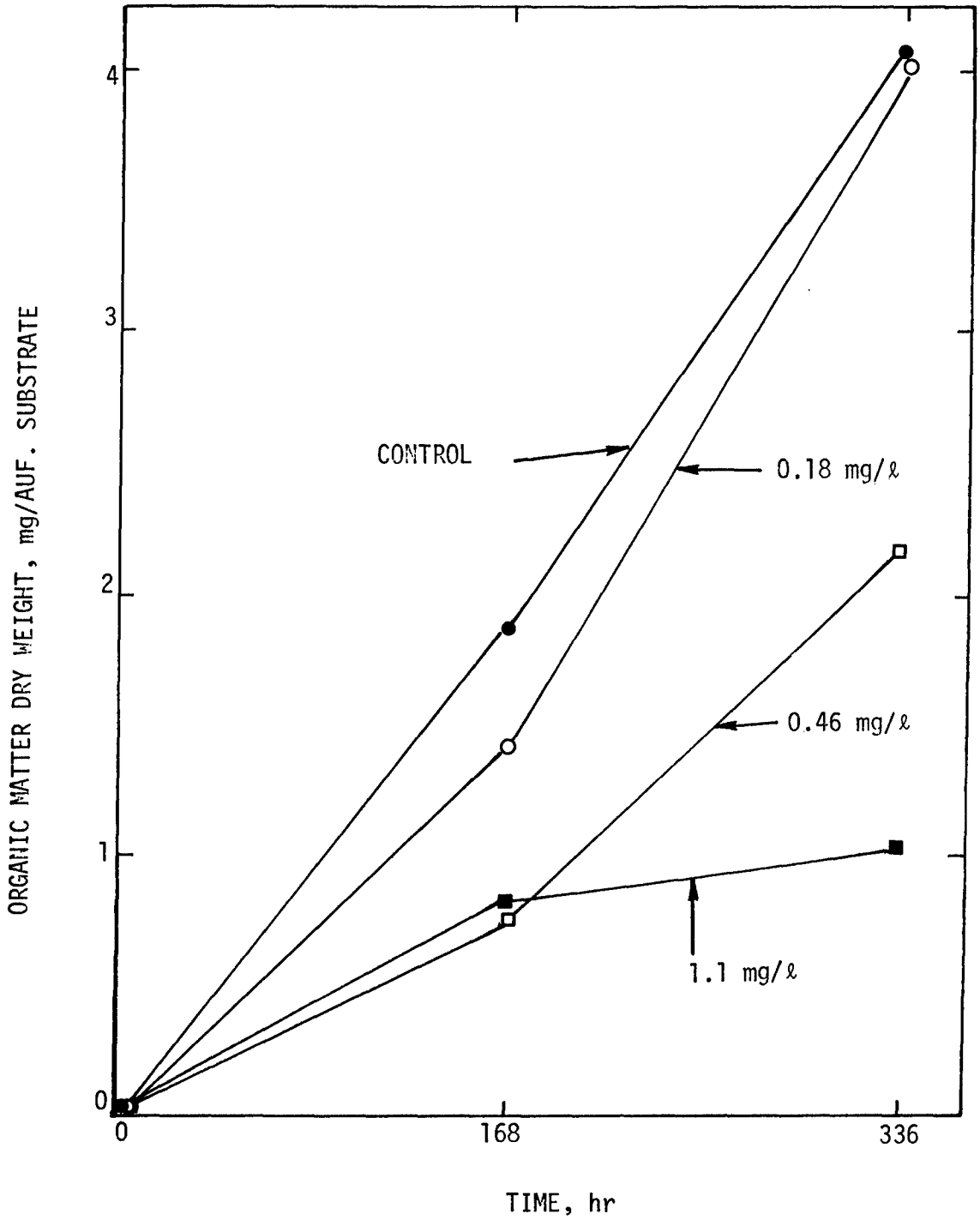


FIGURE 23. EFFECT OF UDMH ON AUFWUCHS ORGANIC MATTER DRY WEIGHT — 336-HR BIOASSAY

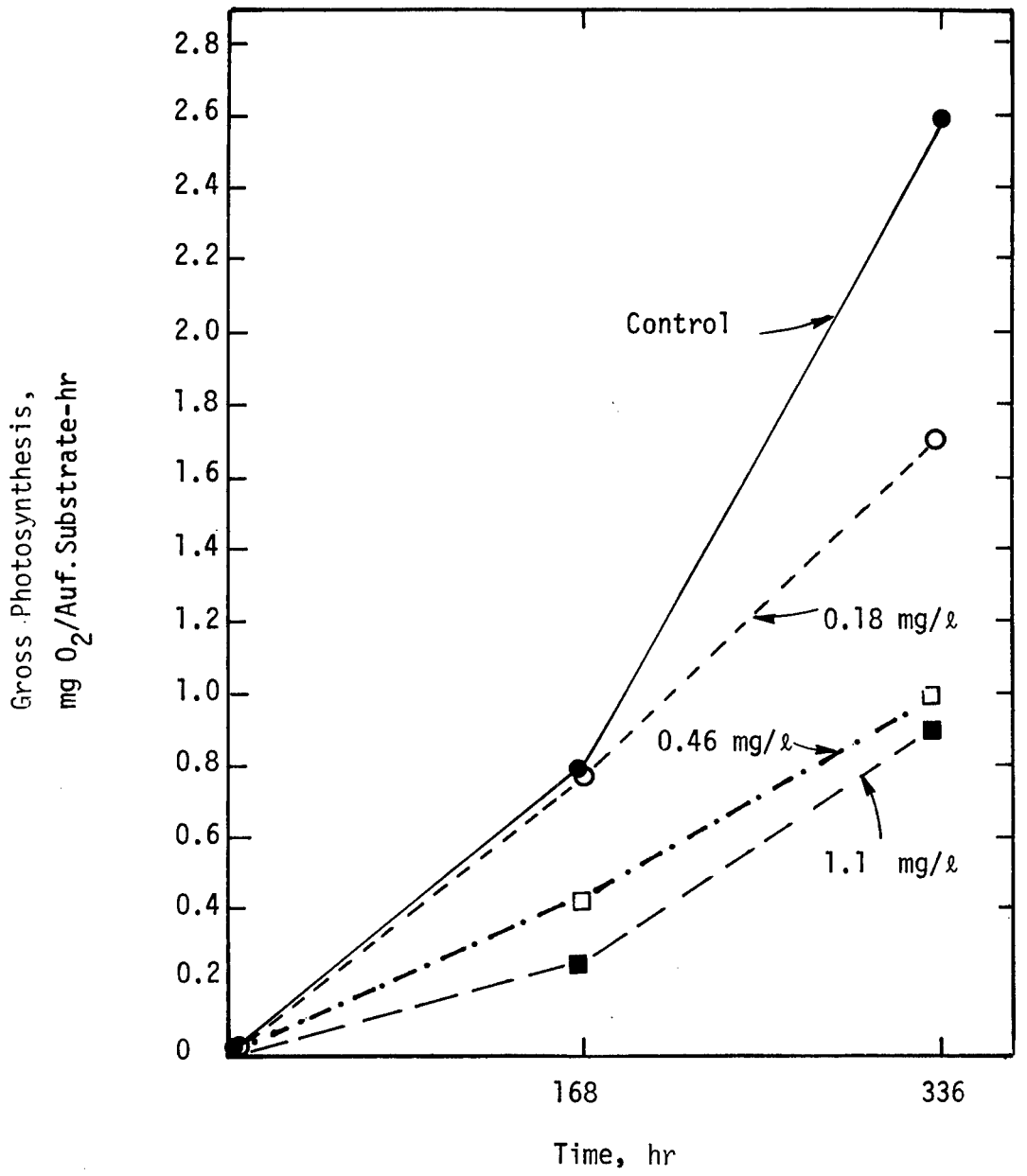


FIGURE 24. GROSS PHOTOSYNTHESIS OF AUFWUCHS EXPOSED TO UDMH 336-hr BIOASSAY

were analyzed on days 0, 7, 14, and 21; UDMH, NH_3–N, pH, and DO were measured on days 0, 4, 8, 12, and 18.

Bay Water Characteristics

Table 26 shows that the Bay water salinity, temperature, and chloride was lower than in the preceding UDMH study.

TABLE 26

PHYSICAL AND CHEMICAL CHARACTERISTICS OF BAY WATER

Constituent	Concentration	
	During Bioassay	During Acclimation Period
Salinity, 0/00	24.5 ± 1.9	22.7 ± 1.9
Temperature, °C	11.8 ± 1.2	9.9 ± 1.4
DO, mg/ℓ	9.7 ± 0.7	
Alkalinity, mg/ℓ	112 ± 10	
Chloride, g/ℓ	11.8 ± 2.4	
pH		8.0 ± 0.4

UDMH, NH_3–N, and pH values are shown in Table 27. The large standard deviations in UDMH levels reflect the difficulty in measuring low UDMH concentrations as well as possibly the effects of heavy rains which may have produced analytical interferences. No differences in NH_3–N and pH were observed in the exposure tanks with the exception of one control, the high ammonia nitrogen content of which is attributed to analytical error.

Stickleback Mortality Results

Table 28 shows that there was an excellent spread of mortalities in this bioassay. The 504-hr LC50 of UDMH was estimated at 0.16 mg/ℓ (95% confidence limits of 0.11–0.22 mg/ℓ). The 336-hr LC50 was estimated (for comparison with the data obtained in the summer bioassay) using the UDMH concentrations and mortalities measured during the period from 0 to 336 hr (Table 28). The value 0.22 mg/ℓ (95% confidence limits of 0.14–0.34 mg/ℓ) is in excellent agreement with the estimated value of 0.2 mg/ℓ obtained in the previous "summer" bioassay. Indeed, the agreement is somewhat surprising in view of the effect of temperature observed on the toxicity of MMH. However, in the experiments on UDMH, both salinity and temperature were lower in the winter bioassay while for MMH only temperature was lower.

Another factor which apparently influenced the MMH summer study results was the high silt content of the sea water which increased mortality in the control fish and likely in the test fish, also.

TABLE 27

UDMH, AMMONIA NITROGEN, AND pH LEVELS IN THE 21-DAY CONTINUOUS FLOW BIOASSAY OF UDMH CONCENTRATION, MEAN ± s

Analog Tank	UDMH*, mg/ℓ			NH_3-N*, µg/ℓ		pH*
	Selected	Measured	Average	Measured	Average	Measured
A	0.0	0.0	0.0	58.5 ±48.6	43.8 ±36.5	7.9 ±0.04
B	0.0	0.0		29.1 ±10.1		7.9 ±0.1
I	0.056	0.048±0.042	0.044±0.039	36.6 ±17.6	36.0 ±17.7	8.0 ±0.1
J	0.056	0.039±0.040		35.4 ±19.9		8.0 ±0.1
K	0.10	0.10 ±0.068	0.10 ±0.068	31.4 ±7.30	29.1 ±7.86	8.0 ±0.1
L	0.10	0.11 ±0.074		26.8 ±8.52		8.0 ±0.1
C	0.32	0.22 ±0.16	0.20 ±0.15	34.6 ±9.80	31.4 ±9.8	7.9 ±0.1
D	0.32	0.19 ±0.15		28.3 ±9.81		8.0 ±0.04

*n = 5

TABLE 28

TOXICITY OF UDMH TO STICKLEBACK

Analog Tank	UDMH Conc. mg/ℓ	Number of Test Organisms	Cumulative Number of Test Organisms Dead After Hour: 24	48-168	192	216	240	264	288	312	336	360-408	432	456	480	504	% Mortality
A	0.0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	0.0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I	0.048	20	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5
J	0.039	20	0	0	0	0	0	0	0	0	1	1	1	1	1	1	5
K	0.10	20	1	1	1	1	1	1	2	2	3	3	3	3	3	3	15
L	0.11	20	0	0	0	0	0	0	0	0	0	0	0	0	0	2	10
C	0.22	20	0	0	2	5	13	16	19	19	19	19	19	20	20	20	100
D	0.19	20	0	0	2	3	4	5	5	5	5	5	6	6	6	6	30

DISCUSSION

INTRODUCTION

From the results of current and previously reported work, the relative toxicity of the jet fuels JP-4, JP-8, and JP-9 components RJ-4, RJ-5, and MCH (methylcyclohexane) to flagfish and rainbow trout can be assessed and the impact of each fuel on the environment evaluated. Similarly, the relative toxicity of MMH, UDMH, and hydrazine to marine life can be evaluated.

RELATIVE TOXICITY OF JP-8, JP-4, AND JP-9

Sublethal effects of WSF of JP-8 and JP-4 were assessed by measuring the growth (length and wet weight) of two species of fish exposed to WSF of these fuels. It can be concluded that JP-4 is more toxic than JP-8. Flagfish growth was not influenced by the presence of 1.7 mg/ℓ WSF of JP-8; however, 1.5 ± 0.8 mg/ℓ WSF of JP-4 retarded flagfish growth. The predicted no-effect level for WSF of JP-4 on flagfish growth was 0.6 ± 0.2 mg/ℓ.

The no-effect level for WSF of JP-8 on rainbow trout was less than 1.4 mg/ℓ (the lowest level tested); for WSF of JP-4 the no-effect level was 1.1 mg/ℓ. One problem that arises in a discussion of no-effect levels derived from these bioassays is the decline in concentration observed as the assay progressed from the yolk-sac stage (egg basket period) to the fry and adult stage (open tank period). For example, in the bioassay of WSF of JP-4 to rainbow trout the lowest WSF of JP-4 concentration changed from a mean of 0.5 ± 0.2 mg/ℓ during the 24-day egg basket period to 0.2 ± 0.1 mg/ℓ in the open tank period. The drop in WSF concentration is most likely caused by a combination of factors. Alevin are less active than fry which, after swim-up, swim around vigorously in search of food and cause turbulence which enhances loss of WSF by volatilization. In addition, the presence of food and excretion products during the open tank period enhances biodegradation of fuel.

Because of the change in WSF of fuel concentration, the no-effect concentrations (for fish growth) should be viewed as tentative. They are the lower WSF concentrations observed during the open tank period whereas the effects on growth may be related to the previously higher WSF concentrations to which the eggs were exposed.

Data on egg hatching also suggested that WSF of JP-4 is more toxic than WSF of JP-8. Flagfish fry exposed to 6.6 ± 1.9 mg/ℓ WSF of JP-4 were deformed but a similar concentration of JP-8 (6.8 ± 1.1 mg/ℓ) did not cause deformity. At WSF concentrations of approximately 6 mg/ℓ both fuels had no effect on the hatching success of either rainbow trout or flagfish eggs; but all fry of both types of fish died soon after hatching.

At lower WSF of fuel concentrations, egg hatching showed a species-related response to hydrocarbon fuels. The rate of hatching of flagfish eggs was retarded at 1.7 mg/ℓ WSF of JP-8 and 2.7 mg/ℓ WSF of JP-4. The rate of hatching of rainbow trout eggs was accelerated at 2.1 mg/ℓ WSF of JP-8 and 1.1 mg/ℓ WSF of JP-4.

JP-9 was more toxic than either JP-4 or JP-8 to flagfish eggs. A WSF of JP-9 concentration in excess of 0.23 mg/ℓ reduced hatching success.

This toxicity was ascribed to the RJ-5 component of JP-9 since it was found that an RJ-5 concentration of greater than 0.05 mg/ℓ reduced the hatching success of flagfish eggs.

Rainbow trout survival was influenced by similar WSF of JP-4 and JP-8 concentrations. There was a significant effect on rainbow trout mortality at WSF of JP-8 concentrations between 1.4 and 1.8 mg/ℓ and WSF of JP-4 concentrations between 1.7 and 3.5 mg/ℓ. The effect of these two fuels on flagfish survival is the only evidence contradictory to the general conclusion that JP-4 is more toxic to fish than JP-8. Flagfish mortality was increased by WSF of JP-8 concentrations of between 1.5–3.0 mg/ℓ, but no effect on flagfish mortality was observed at 3.0 mg/ℓ WSF of JP-4.

The lethality of the WSF of JP-9 and its components to rainbow trout was far greater than either the WSF of JP-4 or WSF of JP-8. Nonlethal concentrations were below 0.03 mg/ℓ RJ-4, below 0.04 mg/ℓ RJ-5, below 0.8 mg/ℓ MCH, and below 0.38 mg/ℓ WSF of JP-9.

JP-4 accumulated in the whole body tissue of fish to a greater extent than JP-8. The JP-4 accumulation ratio for flagfish was 270 and for rainbow trout, 170; JP-8 accumulation ratios were 160 for flagfish and approximately 85 for rainbow trout. These results may reflect the relatively higher proportion of low molecular weight components in WSF of JP-4. These lower molecular weight components are more readily sorbed into tissue than are the predominantly higher molecular weight components in WSF of JP-8.

MCH accumulated to a similar extent as the WSFs of JP-4 and JP-8 in flagfish and rainbow trout; the accumulation ratio was 150. However, both RJ-4 and RJ-5 accumulated to much greater extents – rainbow trout concentrated RJ-4 9800 times and RJ-5 3900 times.

Specific tissue analysis indicated a 2.7-fold greater accumulation of WSF of JP-8 in flagfish liver than in whole body tissue. WSF of JP-4 accumulated about 3 times as much in golden shiner liver as in muscle tissue and nearly 20 times as much in the GI tract as in muscle tissue.

While depuration rates are not directly comparable, they indicate a 90% depuration of WSF of JP-8 from flagfish whole body tissue after 14 days and nearly 100% depuration of WSF of JP-4 from golden shiner innards after 17 days. These data were derived from fish exposed to approximately 1 mg/ℓ WSF of fuel. When the fuel was accumulated from a WSF of JP-4 of 2.5 mg/ℓ, there was an approximately 75% removal from innards after 17 days depuration. MCH was almost completely voided in 12 hr from rainbow trout tissue, but RJ-4 and RJ-5 were completely retained in the tissue after 8 days depuration.

Based on the preceding comparisons, it is apparent that deleterious effects on fish of WSF of fuels are much more severe for JP-9 than for either JP-4 or JP-8. The RJ-5 component, in particular, appeared to cause the most damage to fish. This component separated out from the fuel mixture when it was subjected to a turbulent water stream. Globules of RJ-5, which is denser than water, can be expected to be deposited on the bottom of streams after a spill and present a more permanent environmental hazard than either

WSF of JP-4 or JP-8 which are lighter than water and hence would be dissipated after a spill.

The preponderance of evidence demonstrated that WSF of JP-4 was more toxic to fish than WSF of JP-8. The differences observed were slight but statistically significant and would be expected to exert a significant difference in the environment. As expected, rainbow trout proved much more sensitive to the fuels than flagfish.

The impact of changes in rate of egg hatching at fuel concentrations on the order of 1-2 mg/ℓ may be important. The acceleration of rainbow trout egg hatching is not accompanied by corresponding hastening of growth. On the contrary, the alevin are significantly smaller on swim-up day than the controls, indicating a deleterious effect at an early stage.

Accumulation of fuels in fish tissue occurred at all aqueous fuel concentrations measured. Thus, the presence of even very low levels of fuel in a body of water would have some impact on fish (e.g. causing off tastes and flavors). At the moderately low aqueous fuel concentrations tested, significant levels of fuels accumulated in fish tissues. Thus, for golden shiners exposed to 2.5 mg/ℓ WSF of JP-4 for 17 days it can be estimated that for a typical fish:

a. Accumulation of fuel in the liver would represent 0.057% of the total wet weight of the liver;

b. Accumulation of fuel in the innards would represent 0.34% of the total wet weight of the innards;

c. Accumulation in the muscle would represent 0.018% of the total wet weight of the muscle;

d. Accumulation in the whole body would represent 0.1% of the total wet weight of the fish.

RELATIVE TOXICITY OF THE HYDRAZINES

Static bioassays with stickleback and continuous-flow bioassays with stickleback and aufwuchs indicate that the order of toxicity of the hydrazines is MMH > UDMH > H. As shown in Table 29, stickleback 336-hr LC50s were 0.011 mg/ℓ for MMH, 0.22 mg/ℓ for UDMH, and 1.07 mg/ℓ for hydrazine.

In jar tests utilizing 24-hr renewal of the compounds, the 96-hr LC50 values were substantially higher, i.e., 0.36 mg/ℓ for MMH, 1.6 mg/ℓ for UDMH, and 3.4 mg/ℓ for H. In "spill" tests in which there was no renewal of the compounds, the no-effect levels increased to 1.0-3.2 mg/ℓ for MMH, 3.2-10.0 mg/ℓ for UDMH, and 3.2-5.6 mg/ℓ for H. In both the jar tests and spill tests the hydrazine compounds disappeared rapidly from sea water. This factor explains the increase in LC50 values for stickleback in these experiments.

Stickleback exposed to hydrazine exhibited stressed behavior such as disorientation in swimming and increased respiration rate prior to being killed; no stress symptoms preceded mortality in stickleback exposed to MMH or UDMH.

TABLE 29

TOXICITY OF THE HYDRAZINES TO STICKLEBACK

(mg/ℓ)

Compound	Jar Test (24-hr Renewal) 96-hr LC50	Analog 96-hr "Spill" (No Renewal) No Effect Level	Analog Continuous Flow (Continuous Renewal) 336-hr LC50	95% CL
H	3.4	3.2-5.6	1.07	0.78--1.47
UDMH	1.6	3.2-10.0	0.22	--
MMH	0.36	1.0-3.2	0.011	0.003-0.024

Aufwuchs results are summarized in Table 30. The no-effect level of MMH was between 0.01 and 0.15 mg/ℓ, for UDMH the no-effect level was between 0.18 and 0.46 mg/ℓ, and for H the no-effect level was between 0.17 and 0.52 mg/ℓ. The order of toxicity of the hydrazines was different in "spill" situations: H was the most toxic compound. These results are not inconsistent with the continuous flow data because they reflect the relative rate of decay of the three materials. H decays much more slowly than UDMH and MMH. In the absence of renewal, the actual concentration of hydrazine after 24 hr is about 4 times larger than that of either MMH or UDMH.

TABLE 30

TOXICITY OF THE HYDRAZINES TO AUFWUCHS

(mg/ℓ)

Compound	Analog 96-hr "Spill" (No Renewal) No Effect Level	Analog 336-hr Continuous Flow (Continuous Renewal) No Effect Level
H	0--3.2	0.17-0.52
UDMH	3.2-10.0	0.18-0.46
MMH	> 3.2	0.01-0.15

From an environmental standpoint, a spill of H would be more serious to aufwuchs than a comparable spill of either MMH or UDMH. However, stickleback are more sensitive to a spill of MMH than of hydrazine.

The diversity of species in control aufwuchs was greater than in aufwuchs exposed to the hydrazines. There appeared to be a change from an autotrophic to a heterotrophic community possibly due to inhibition of photosynthetic species.

Of the many indicators of toxicity to aufwuchs examined (Chlorophyll *a*, *b*, *c*, pheophytin *a*, organic content, aufwuchs dry weight, gross photosynthesis, and photosynthetic indices with respect to weight and Chlorophyll *a*)

the most sensitive and reliable indicator appeared to be gross photosynthesis. Other indicators often provided a confusing pattern of results. In some instances, sparse aufwuchs growths compounded the difficulty of interpreting results from indicators related to the weight of the standing crop. Inconsistent growth was caused by seasonal fluctuations in salinity, temperature, and possibly nutrients. Low nutrient levels and low salinity have been reported to cause stress in aufwuchs communities (Krock and Mason, 1971) and affect biological communities (Pearson et al., 1970).

The relative sensitivity of crabs, mussels, and stickleback to hydrazines, is summarized in Table 31. The crab species, Hemigrapsus oregonensis, was more sensitive to H than stickleback; mussels were less sensitive than stickleback.

TABLE 31

RELATIVE TOXICITY OF THE HYDRAZINES TO CRABS, MUSSELS, AND STICKLEBACK CONTINUOUS FLOW BIOASSAYS

HYDRAZINE

Test Animal	96-hr LC50, mg/ℓ	336-hr LC50, mg/ℓ
Stickleback	5.4	1.07
Crab (H. oregonensis)	3.6	0.56
Mussel	>5.7	>1.72

MMH

Test Animal	336-hr LC50, mg/ℓ
Stickleback	0.011
Crab (H. nudus)	0.012-0.15
Mussel	0.012-0.15

UDMH

Test Animal	336-hr LC50, mg/ℓ
Stickleback	0.2
Crab (H. nudus)	0.2-0.46
Mussel	0.2-0.46

Stickleback were more sensitive to MMH and UDMH than either the crab species Hemigrapsus nudus or mussels. Based on the rate of mortality during the 336-hr bioassay H. nudus was judged to be more sensitive to MMH than mussels; the reverse was the case for UDMH.

APPENDIX 1

ANALYTICAL PRECISION OF GAS CHROMATOGRAPHIC DETERMINATIONS OF JP-8

Day	mg/ℓ	Mean ± s
-2	10.9, 10.3	10.60 ± 0.42
7	11.5, 10.5	11.00 ± 0.71
11	10.2, 10.2	10.20 ± 0.00
12	9.9, 10.1	10.00 ± 0.14
13	10.4, 10.7	10.55 ± 0.21
19	9.1, 9.1	9.10 ± 0.00
20	9.8, 11.0	10.40 ± 0.85
28	11.3, 10.9	11.10 ± 0.28
32	10.6, 10.6	10.60 ± 0.00
35	10.1, 10.4	10.25 ± 0.21
40	10.5, 9.9	10.20 ± 0.42
48	9.7, 9.8	9.75 ± 0.07
49	10.9, 10.6	10.75 ± 0.21
54	11.0, 12.8	11.90 ± 1.27
55	9.3, 9.8	9.55 ± 0.35
56	13.4, 11.9	12.65 ± 1.06
61	10.0, 9.9	9.95 ± 0.07
67	11.2, 10.8	11.00 ± 0.28
75	12.3, 10.8	11.55 ± 1.06
82	9.6, 9.1	9.35 ± 0.35
90	10.3, 9.5	9.90 ± 0.57
105	11.5, 10.3	10.90 ± 0.85
110	9.6, 9.6	9.60 ± 0.00

n = 23 pairs

mean of means = 10.47

s of s = 0.38

APPENDIX 2

PURGE-AND-TRAP QUANTITATION OF JP-4

Fuel Age days	WSF of JP-4 Concentration mg/ℓ	Mean ±s mg/ℓ
1	24.80	
1	27.64	
1	22.38	
1	17.81	22.94 ± 3.1
1	21.70	
1	19.34	
1	20.84	
1	26.75	
1	23.70	
2	24.40	
2	15.68	
2	19.68	20.00 ± 3.6
2	20.23	
3	24.51	
3	17.39	20.95 ± 5.0
4	20.18	
4	23.24	18.66 ± 5.0
4	15.10	
4	16.10	
Overall (all fuel ages)		20.64 ± 1.8

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