APPENDIX F-1

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BROOD SIZE AND GROWTH OF NEOMYSIS MERCEDIS

Final Report

25 September 1985

Prepared for:

CAIFORNIA STATE WATER RESOURCES CONTROL BOARD
301 P Street, Third Floor
Sacramento, California

Attention: Dr. John Cornacchia

Submitted by:

Howard C. Bailey, Director
Aquatic Toxicology Program

SRI Project LSU-7578

Approved by:

Jøn B. Reid, Director
Toxicology Laboratory

W. A. Skinner, Vice President
Life Sciences Division

SRI International®

333 Ravenswood Ave. • Menlo Park, CA 94025
415 326-6200 • TWX: 910-373-2046 • Telex: 334-486

Introduction

Neomysis mercedis is a small crustacean relatively widely distributed throughout the Sacramento-San Joaquin Delta and also found in other locations, primarily estuaries along the Pacific Coast from Southern Alaska to Gaviota, California (Holmquist, 1973; Orsi and Knutsen, 1979a). In the Delta, it is considered to be the primary food supply for juvenile fishes including the striped bass (Morone saxatilis) (Heubach, 1972; Orsi and Knutsen, 1979b). Because of its appreciable importance as a component of the food web, interest has focused on Neomysis regarding potential effects of environmental perturbations, including changes in water use and quality and the effects of chemical contaminants. Unfortunately, much of the basic information needed to characterize neomysid populations, such as data on growth and reproduction, is not available because this species is difficult to maintain under laboratory conditions. This paper presents data on brood size and growth in N. mercedis.

Methods and Materials

Neomysids were originally obtained from Montezuma Slough in December 1982 and have since been under continuous culture. Cultures have maintained in 15-gal glass aquaria at 16 to 20°C. The neomysids were fed brine shrimp nauplii and were maintained at a salinity of approximately 3500 µmhos. A photoperiod of 16 hr L-8 hr D, with a light intensity of about 60 ft candles, was used.

To determine the brood size of the neomysids, gravid females (12 to 14 mm) were selected from the culture tanks and placed in 75 × 150-mm glass crystallizing dishes which were maintained under continuous-flow conditions. Each dish was checked daily for young and any present were counted and removed. The hatching process was determined to be complete when a moulted exoskeleton from the female was observed in the dish.

Growth rates were determined by transferring newly hatched young to 3-L battery jars containing No. 20 sand and airlift pumps to circulate the water. The young neomysids were maintained at 17 to 18°C and were fed Artemia nauplii. Lighting and water quality were the same as in the

culture tanks. At different time periods, neomysids were removed from the jars and measured. Small neomysids were placed in a depression slide over a millimeter scale on a dissecting microscope equipped with a Polaroid camera. The neomysids were lined up with the scale and several photographs were taken so lengths could be determined later. When the neomysids became too large for the depression slide, they were placed on a light box in small dishes backed by millimeter grids and photographed with an Olympus 35 mm camera equipped with extension tubes for close-range photographs. All measurements taken were from the end of the telson to the anterior symphysis of the eyestalks.

Several models were fitted to the growth data. The best fitting model was of the form:

$$\text{Length} = \beta_2 \frac{e^{(\beta_0 + \beta_1 \cdot \text{age})}}{1 + e^{(\beta_0 + \beta_1 \cdot \text{age})}} \quad \text{(age is in days)} \quad .$$

This model was fitted to the data with the NUN procedure in SAS. the values at hatch were included with a time of 0.01 days. With length in mm and age in days, the best fitting coefficients were:

$$\beta_0 = -01.553 \pm 0.064$$
$$\beta_1 = 0.082 \pm 0.005$$
$$\beta_2 = 14.221 \pm 0.314$$

with a residual mean squared error of 0.1382, corresponding to a residual standard error of 0.3717 mm.

Results

Young were counted from a total of 28 broods. The average size of the broods was 15.1 young with a standard deviation of 7.1. Brood sizes ranged between 4 and 31 young. The frequency distribution of brood size is shown in Figure 1. As indicated in the figure, the distribution of young per brood was fairly broad, with no tight cluster around any particular brood size: 71% of the broods contained between 8 and 22 young and, of the total young produced in the study (423), 69% resulted from broods containing 8 to 22 young.

An interesting observation occurred during the study when it was noted that it took up to three days for adult neomysids to give birth. Brood size was then plotted against the time required to complete hatching in an effort to determine if larger broods were associated with longer delivery times. This plot is shown in Figure 2.

Figure 1. Frequency Distribution of Number of Young per Brood in N. mercedis (N = 28)

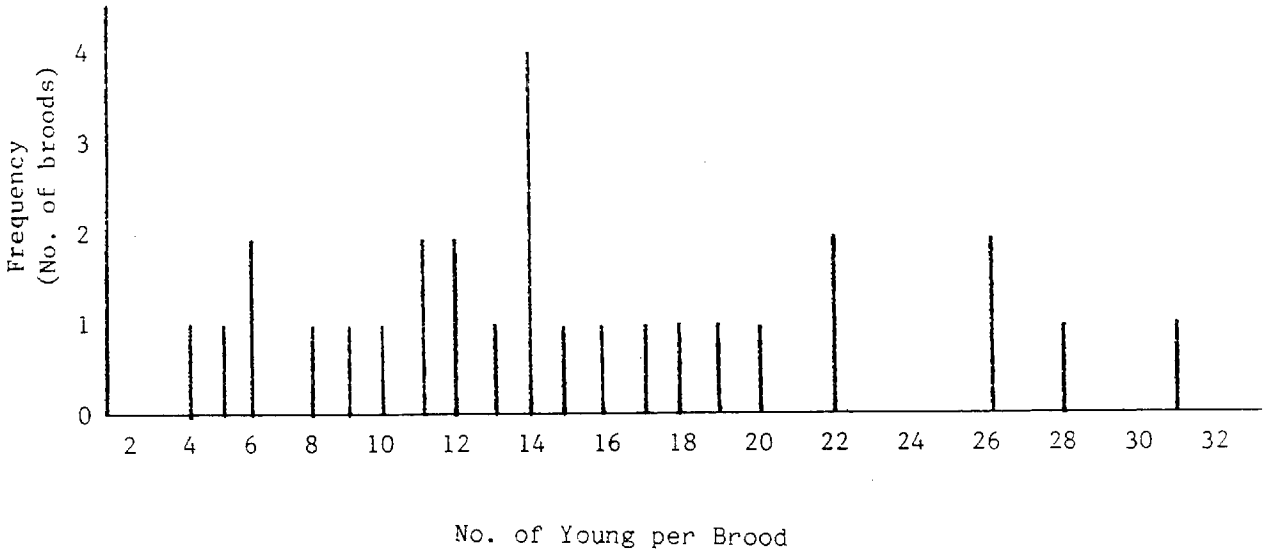
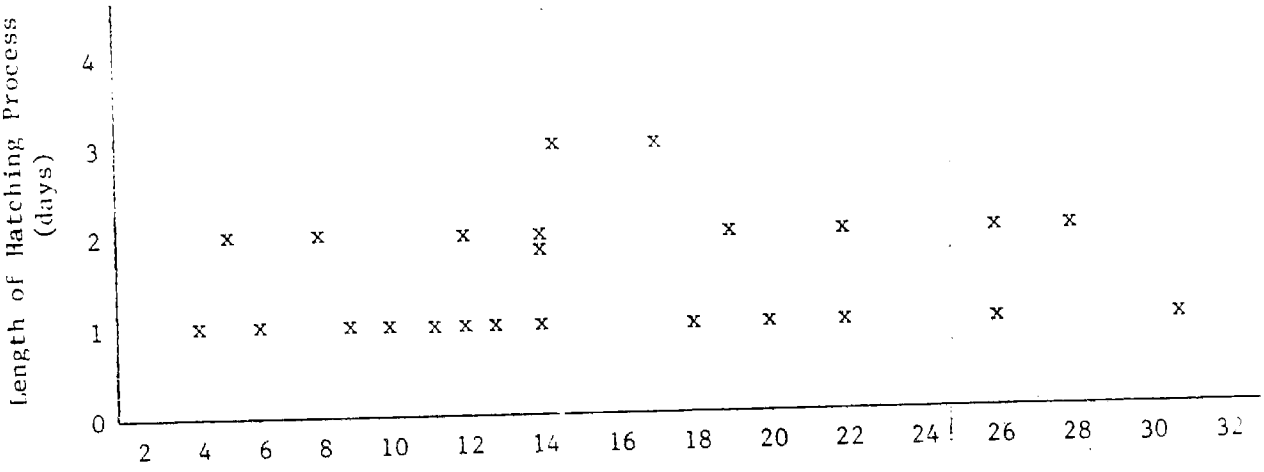


Figure 2. The Relationship Between Brood Size and Hatching Time in N. mercedis (N = 28)



As indicated by the figure, there appears to be no linear relationship between brood size and the time required to complete the hatching process. Of the total of 28 broods, 54% were hatched in 1 day, 39% were hatched in 2 days, and 7% required three days to complete the process.

The growth data and the associated fitted curve with $\pm 2\sigma$ limits on individual variation are shown in Figure 3. The fitted curve and the confidence limit relationships were inverted to obtain predictions of age based on length. These values are summarized in Table 1, which shows the best estimate of age and a confidence region for age for a given length. Photographs of the neomysids at different ages are shown in Figure 4.

Figure 3

MYSID LENGTH VERSUS TIME, WITH FITTED CURVE

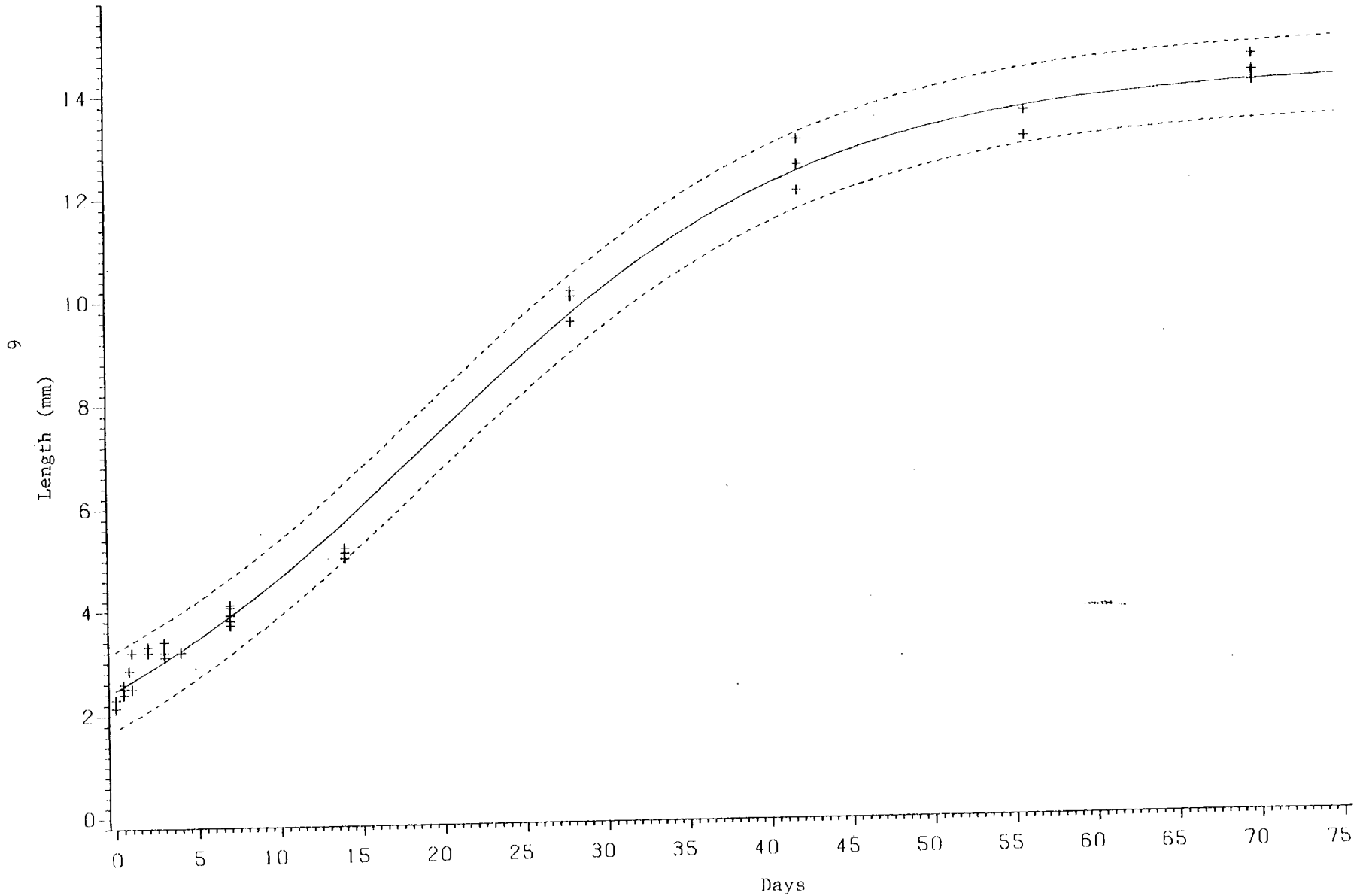


Table 1

PREDICTED AGE (DAYS) OF NEOMYSIDS
AS A FUNCTION OF LENGTH

Length (mm)	Estimated Age (Days)	Confidence Interval for Estimated Age (Days)	
		Lower Limit	Upper Limit
1.5	0.0	0.0	0.0
2.0	0.0	0.0	3
2.5	0.1	0.0	5
3.0	3	0.0	7
3.5	5	0.1	9
4.0	7	3	11
4.5	9	5	13
5.0	11	7	15
5.5	13	10	17
6.0	15	11	18
6.5	17	13	20
7.0	18	15	22
7.5	20	17	24
8.0	22	18	25
8.5	24	20	27
9.0	25	22	29
9.5	27	24	31
10.0	29	25	34
10.5	31	27	36
11.0	34	29	39
11.5	36	31	43
12.0	39	34	47
12.5	43	36	54
13.0	47	39	68
13.5	54	43	*
14.0	69	48	*
14.5	*	54	*
15.0	*	69	*

*> 75 days.

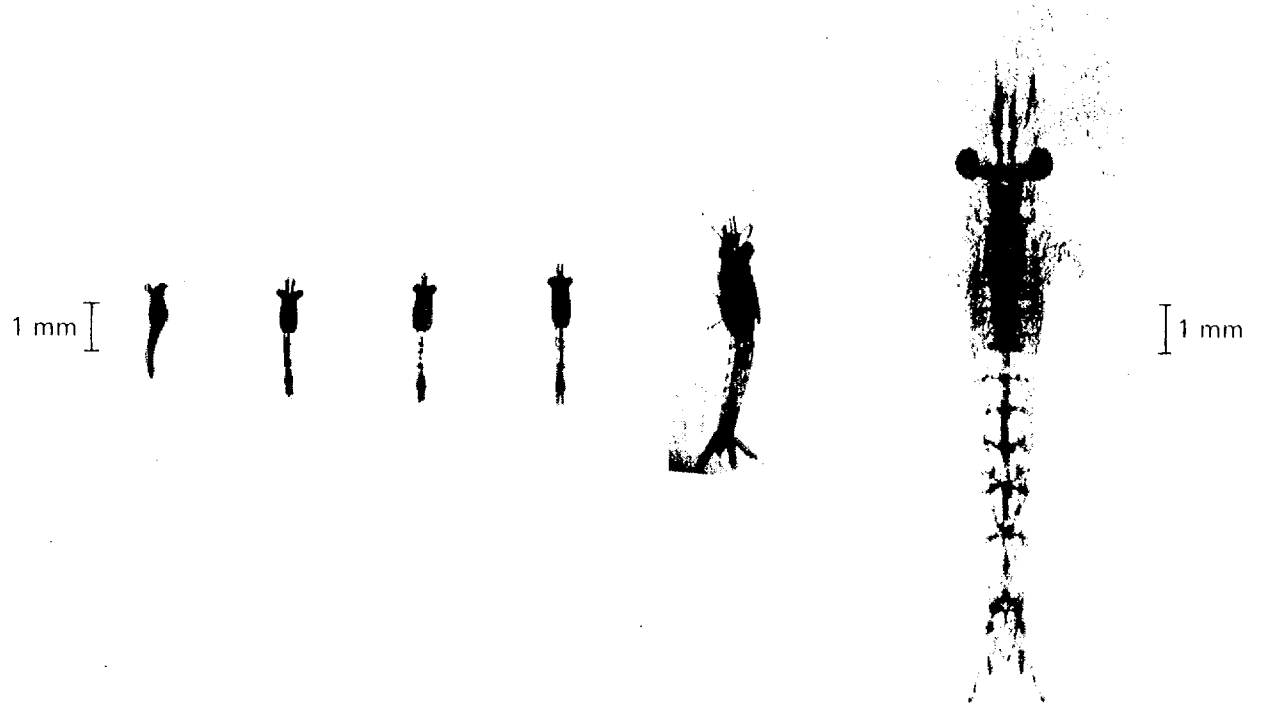


FIGURE 4. N.MERCEDIS AT DIFFERENT AGES.

Discussion

Relatively little data appears to be available on the reproductive capacity of Neomysis mercedis and is limited to analysis of the number of eggs found in the marsupium of preserved specimens. Heubach (1969) reported that the number of eggs present in female neomysids was positively related to the size of adult and also varied seasonally. For females ranging in size from 7 to 17 mm, the number of eggs present ranged between 5 and 55. Additional detail is provided by Siegfried et al. (1979) who found that females approximately 7 mm in length contained 6 to 22 eggs, whereas females measuring 14 mm contained 36 to 65 eggs. These investigators also reported seasonal variations in the number of eggs present as well as an increase in fecundity with increased length.

In comparing the number of eggs found in the brood pouches by other investigators with the number of young actually produced in our study, it is apparent that actual brood size is appreciably less than the number of eggs found in the pouch. Brood sizes produced by females in this study ranged between 4 and 31 young compared with 36 to 65 eggs found in the pouches of 14-mm females by Siegfried et al. (1979). This difference could be due to resorption or loss of eggs prior to hatch or to reduced fecundity related to the captive state of the adults. Preliminary data suggests that the former explanation is more likely, as dissections of gravid females from our cultures revealed the presence of more eggs than typically seen in broods. This has implications from a management point of view in that population estimates based on size of the females and number of eggs present are likely to be substantially higher than the actual number of young produced. In addition, the large variability in the number of young (or eggs) produced in a given size class of female neomysid reduces the precision of any such estimates (i.e., the smallest and largest broods in our study of similarly sized female neomysids differed by a factor of about 8).

Heubach (1969) suggested that the female neomysids die after spawning although Wilson (1951) concluded, on the basis of size frequencies in field populations, that some females reproduced at least twice. Our

observations indicate that, given satisfactory water quality, only about 20 to 30% of the females died after the first broods, and the survivors were capable of reproducing again.

Data on growth in N. mercedis appears limited to descriptions of size ranges found in field samples. Heubach (1969) and Kost and Knight (1975) found a maximum size of 17 mm in their specimens, while Holmquist (1973) reported a maximum size of 19 mm for N. mercedis. Although larger specimens were present in the culture aquaria, neomysids in the growth study only achieved a length of 14 to 15 mm after a 70-day period.

Data on growth rates are exceedingly sparse. Based on size distributions in field data, Wilson (1951) inferred that the average size of N. mercedis increased from 5.8 to 7.2 mm during a 4-month period from winter to spring. He also suggested that a population averaging 9.3 mm in October had been born in May and June. These growth rates are much slower than we obtained in the laboratory. For example, cultured neomysids increased from 5.5 to 7.5 mm in about 7 days compared with 4 months, and went from birth to 9.5 mm in 27 days again compared with 4 months. Wilson (1951) also indicated that growth to maturity takes about one year; broods were produced in the laboratory by 60- to 80-day-old neomysids. The lower growth rates reported by Wilson may be due to environmental factors not present in the laboratory study. For example, temperatures at the time of the field collections ranged between 3 and 12°C compared with 16 to 19°C during the laboratory study. It is well-known that growth of poikilotherms is highly influenced by temperature, with higher growth rates occurring at higher temperatures within the organisms' tolerance range. In addition, the laboratory specimens were provided with a continuous supply of high-quality food, something that is not always present under field conditions. Thus, higher temperatures and food supply would likely account for much of the observed differences in growth. Because the differences are so large, caution should be exercised when applying these age-growth curves to neomysids occurring in temperature regimes markedly different from the one used in this study. Obviously, development of different growth curves at different temperatures and/or feeding levels

would aid in developing size-age relationships for neomysids in different areas in the field.

Acknowledgments

Anne Tait, Paul Haskins, Kevin Joe, and Jesse Martin all assisted in maintaining the cultures and performing the studies. Their assistance is greatly appreciated.

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APPENDIX F-2

SRI International

ACUTE TOXICITY OF RICE HERBICIDES TO NEOMYSIS MERCEDIS

Final Report

19 July 1985

Prepared for:

STATE WATER RESOURCES CONTROL BOARD
901 P Street, Third Floor
Sacramento, California 95801

Attention: John Cornacchia, Ph.D.
Environmental Specialist

Submitted by:

Howard C. Bailey

Howard C. Bailey, Director
Aquatic Toxicology Program

SRI Project LSU-7578

Approved by:

Jon B. Reid

Jon B. Reid, Director
Toxicology Laboratory

W. A. Skinner

W. A. Skinner, Vice President
Life Sciences Division

SRI International®

333 Ravenswood Ave. • Menlo Park, CA 94025
415 326-6200 • TWX: 910-373-2046 • Telex: 334-486

QUALITY ASSURANCE UNIT

Final Report Statement

SRI International assures the quality and integrity of this study, Acute Toxicity Of Rice Herbicides To Neomysis Mercedis, for the State Water Resources Control Board.

The study was inspected on August 2, 1985 during the 50 day chronic termination phase. The findings of the Quality Assurance Unit inspection were reported at the time of the inspection to the Study Director. SRI management was informed of the inspection results on August 5, 1985. A data audit was performed on August 29, 1985. The Study Director and SRI management were informed of the audit results on August 29, 1985.

The final report was audited and reviewed on September 25, 1985. The results of the final report review were communicated to the Study Director and SRI management on September 26, 1985. The final report accurately describes the methods and standard operating procedures and reflects the raw data of the study. Any deviations from the approved protocol and standard operating procedures were made with proper authorization and documentation.

Quality Assurance Director

11/26/85
Date

CONTENTS

INTRODUCTION

At the request of the State Water Resources Control Board, SRI International performed flow-through, acute-toxicity studies on rice herbicides, using Neomysis mercedis as the test organism. The objective of the study was to provide data on the acute toxicity of rice herbicides to organisms inhabiting San Francisco Bay and Delta. Testing was initiated on 22 December 1984 and completed on 1 March 1985.

Copies of this Final Report and the laboratory notebook containing the raw data for this study will be retained in Building 253, Room C-2, for one year from the date of the Final Report. Thereafter, these materials will be stored in SRI's Records Center.

The Study Director on this project was Howard C. Bailey, who was assisted by Paul A. Haskins, Kevin Joe, Jesse Martin, and Anne Tait, biological technicians.

MATERIALS AND METHODS

Test Chemicals

The test chemicals were technical-grade molinate and thiobencarb. Molinate was obtained from Stauffer Chemical Company and thiobencarb was obtained from Chevron Chemical Company. The purity of the materials was not verified by SRI. The test chemicals were stored in a hood at room temperature at SRI.

Test Organism

The test organisms were neomysid shrimp (Neomysis mercedis). They were obtained from cultures maintained at SRI and originating from stock collected in Montezuma Slough in December 1982. The cultures are housed in 15-gallon glass aquaria containing reconstituted water at 3000 to 4000 µmhos and are fed Artemia nauplii. All mysids appeared to be healthy and feeding well.

Diluent Water

Diluent water was prepared by mixing dechlorinated tap water and artificial seawater to achieve a final conductivity of approximately 3500 µmhos. This water was made in 50-gallon batches and stored at 16 to 19°C in Nalgene containers until use.

Test Procedures

96-Hour Bioassay

Test concentrations of molinate and thiobencarb were based on range-finding studies conducted previously. The nominal test concentrations were 0, 0.10, 0.56, 1.00, 5.6, and 10.0 mg/L for molinate and 0, 0.01, 0.056, 0.10, 0.56, and 1.00 mg/L for thiobencarb. The concentrations were made by mixing appropriate volumes of diluent water and aqueous solutions of the test chemicals in Mariotte bottles, which delivered the test solutions to two replicate 100 mm × 190 mm crystallizing dishes for each concentration. The dishes drained into glass-catch bottles, from which samples were taken for chemical analyses. Test temperatures were maintained between 17 and 18°C and a 16 hr L:8 hr D photoperiod was used.

The tests were initiated by selecting neomysids from the culture tanks and placing them together in a 5-L glass animal jar. They were then

distributed, two or three at a time, to the exposure dishes, using stratified random assortment. While on test (96 hours), the neomysids were fed Artemia nauplii daily. The dishes were inspected daily and any dead mysids and uneaten nauplii were removed. At the conclusion of each assay, the control neomysids were measured.

Time-Independent Bioassays

These bioassays were designed to provide an estimate of the concentrations of herbicides that the neomysids could tolerate indefinitely. The procedures were essentially the same as those described above except that the exposures lasted at least 14 days or until a period of 48 hours passed with no deaths occurring.

In addition, the neomysids were exposed to a mixture of the herbicides to assess the degree of its toxicity. The concentrations for the mixture were based on the results of the 96-hour studies with the individual herbicides, which were added to the mixture in the same ratio as their respective LC50 values. Thus, for any given treatment level, we would expect the solution containing both herbicides to be twice as toxic (assuming additivity) as a solution containing only one herbicide at the concentration at which it was present in the mixture. Test temperatures were maintained between 16 and 20°C.

Water Quality Measurements

Temperature was measured daily in each of the treatment levels. Dissolved oxygen, conductivity, and pH were measured at the beginning and end of exposure in each concentration in the 96-hour studies and twice weekly in the time-independent studies. The instruments used were manufactured by Yellow Springs Instrument Co. (dissolved oxygen and conductivity meters), Orion (pH meter), and Scientific Products (thermometer).

Chemical Analyses

Samples were taken from the test solutions at 24, 48, and 96 hours for the 96-hour study and twice weekly for the time-independent studies. Samples were placed in amber bottles with Teflon-lined caps and refrigerated until extraction and analysis. The analytical procedures follow.

Thiobencarb

An aliquot of sample, ranging in volume from 100 to 750 ml, was decanted into an appropriately sized separatory funnel and extracted with two portions of dichloromethane (DCM). The volume of DCM depended on the volume of sample used. It was found that a 1:8 ratio worked best (DCM:sample). For example, if 400 ml of sample was to be extracted, then

two 50-ml portions of DCM were used. A 1.0 ml portion of isooctane (2,2,4-trimethylpentane) was added to the combined extracts as a keeper. This solution was concentrated to a volume of approximately 5 ml, using a rotary evaporator at a bath temperature not to exceed 35°C. This concentrated extract was transferred to an 8-ml test tube; the rotary evaporation flask was rinsed twice with DCM bring the volume to approximately 8 ml. This solution was further concentrated, under nitrogen gas, to a final volume of 1.0 ml. Enough internal standard, 3-amino-2,4-DNT (in DCM), was added so that "mg on column" values for 3-amino-2,4-DNT and herbicide were roughly equivalent.

Molinate

A 200-ml portion of sample was decanted into a 250-ml separatory funnel and extracted twice with 25-ml portions of DCM. The extracts were pooled and an appropriate amount of 3-amino-2,4-DNT was added as internal standard.

All samples were analyzed using gas chromatography (Varian Model 3700) with TSD detection (nitrogen-phosphorous specific). An HP3390 integrator was used along with a Supercoport SP2250, 100/120, column. Injection volumes ranged from 1 to 5 µl at 205°C isothermal. Gas flow rates were: air, 175 ml/min; H_2, 30 ml/min; and N_2, 30 ml/min.

Statistical Methods

Determination of LC50

To estimate the median lethal concentration (LC50), we used a computerized program developed at SRI, which is composed of several statistical methods for estmating LC50s. For this project, we used estimates derived from the binomial method when there were no partial responses and estimates from the probit method when there were partial responses.

The binomial method is valid regardless of the form of the underlying tolerance distribution and therefore gives statistically valid, but conservative, confidence intervals in all cases. It is the only appropriate method when a data set contains no partial responses. The method is a two-step process. In the first step, at each concentration level with an observed mortality of 50% or more, a significance level is computed for the hypothesis that the true mortality at that concentration is 50% or less, using only the observations at that concentration. In the second step, at each concentration level with an observed mortality of less than 50%, a significance level is computed for the hypothesis that the true mortality at that concentration is 50% or more. An estimate of the LC50 is derived as the geometric average of the adjacent concentrations with 0 and 100% mortality. The 95% confidence interval for the LC50 is the shortest interval (with limits at the concentrations or at plus or minus

infinity), such that at the upper confidence limit and all higher concentrations, 50% or more of the animals have died and the significance level is 0.025 or less, and at the lower confidence limit and all lower concentrations, less than 50% of the animals have died and the significance level is 0.025 or less.

The probit method is a parametric technique that depends on the assumption that the tolerance of the organisms to the test material follows a normal distribution. The computer routine performs the probit analysis twice--once for the concentration levels expressed in linear units and once for the concentrations expressed in logarithmic units. In either case, Berkson's adjustment (one-half of a response at the highest concentration with no response and one-half of a nonresponse at the lowest concentration with 100% response) is used when there is only one partial response.

The LC50 estimate is the maximum likelihood estimate for the mean of the tolerance distribution. The "unadjusted" confidence interval for the LC50 is derived by inverting the likelihood ratio test for determining whether any specified concentration is the LC50. A chi-square test is used to determine how well the estimated tolerance distribution fits the data (which are also plotted). In this test, adjacent concentration levels are collapsed until the expected responses (mortality and survival) are everywhere greater than 2.0. Finally, if the probability of poor fit is 0.75 or greater, a heterogeneity factor is derived from the chi-square test and the confidence interval is adjusted outward using the heterogeneity factor.

Contribution of the Components of the Mixture to the Total Toxicity

To determine whether the toxicity of the mixture of herbicides was more or less than the sum of the toxicities of the individual components, we used a modified version of a method developed by Marking and Dawson.[1] These authors calculated the sum (S) of the contributions of two compounds (A and B) to the toxicity of a mixture of the two compounds using the equation:

$$\frac{A_m}{A_i} + \frac{B_m}{B_i} = S \quad ,$$

---

[1]Marking, L. L., and V. K. Dawson. 1975. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals. Investigations in Fish Control Series, Report No. 67, U.S. Department of the Interior, Fish and Wildlife Service.

where A_i and B_i are the individual LC50s of compounds A and B, and A_m and B_m are the LC50s of the compounds in the mixture. The values A_m and and B_m are calculated by multiplying the LC50 of the mixture by the fraction contributed by each compound to the total concentration of both compounds in the mixture.

To explain the logic of Marking and Dawson's equation, we have rewritten it as follows:

$$S = \frac{aLC50_M}{LC50_A} + \frac{bLC50_M}{LC50_B} \quad ,$$

where $LC50_A$, $LC50_B$, and $LC50_M$ are the experimentally derived estimates of acute toxicity for compounds A, B, and the mixture (M), respectively, and a and b are the respective fractions of compounds A and B in the mixture.

If $LC50_A$, $LC50_B$, and the amount of A and B in M are known, the theoretical concentrations of M that would kill 50% of the test organisms (under the assumption that A and B were neither antagonistic nor synergistic) can be calculated. We call that concentration the additive LC50 of M and denote it as $LC50_M$*.

On the basis of acute toxicity tests, each gram of compound B is only $LC50_A/LC50_B$ as toxic as each gram of Compound A. If we assume that the toxicities of A and B are additive, each gram of the mixture M should be as toxic as a + b ($LC50_A/LC50_B$) grams of A. Thus, the additive LC50 of M is:

$$LC50_M^* = \frac{LC50_A}{a + b(LC50_A/LC50_B)} \quad .$$

By algebraic manipulaton, it can be shown that

$$S = LC50_M/LC50_M^* \quad .$$

The statistic S ranges from zero to infinity, with a value of 1.0 denoting additivity. Because the range of S is nonsymmetric around 1.0, Marking and Dawson (1975) suggested replacing S by a corrected sum, which we will call CS, and which these authors defined as CS = 1/S-1 when S < 1 and CS = 1 - S when S > 1.

We find litle merit in this definition and redefine CS as CS = Log S. This transformation symmetrizes the range around zero. A CS value of zero indicates additivity, a CS value of -1 corresponds to $LC50_M$ = $LC50_M$*/10 (or synergism), and a CS value of +1 corresponds to $LC50_M$ = 10 ($LC50_M$*) (or antagonism). This transformation also simplifies the derivation of the confidence interval for CS.

Marking and Dawson (1975) derived the 95% confidence interval for CS by substituting the 95% confidence intervals for LC50, $LC50_B$, and $LC50_M$ into their equation for calculating S. We believe this procedure to be heuristic because the 95% confidence interval for CS (i.e., Log S) can be rigorously defined using the procedure described below. From the above equation,

$$\text{Log } S = \text{Log } LC50_M - \text{Log } LC50_M^* \quad ,$$

if the central limit theorem can be invoked (e.g., if Log $LC50_M$ and Log $LC50_M^*$ can be assumed to be normally distributed), then an approximate 95% confidence interval for the true corrected sum can be calculated by:

$$\text{Log } S \pm 1.96[\text{VAR}(\text{Log } LC50_M) + \text{VAR}(\text{Log } LC50_M^*)]^{1/2} \quad ,$$

where VAR denotes variance. The variance of Log $LC50_M$ can be estimated from the results of the toxicity test on the mixture. The variance for Log $LC50_M^*$ can be estimated by the equation:

$$\text{VAR}(\text{Log } LC50_M^*) = \left(\frac{bLC50_A}{aLC50_B + bLC50_A} \right)^2 \text{VAR}(\text{Log } LC50_B)$$

$$+ \left(\frac{aLC50_B}{aLC50_B + bLC50_A} \right)^2 \text{VAR (Log } LC50_A)$$

RESULTS

Acute Studies

The mortality of neomysids exposed to thiobencarb for 96 hours is summarized in Table 1. The 96-hour LC50 estimate of approximately 0.3 mg/L indicates that thiobencarb is relatively toxic to neomysids. In addition, the decreasing trend in LC50 values over the course of exposure suggests that the toxicity is cumulative in nature.

Table 1

MORTALITY OF NEOMYSIDS EXPOSED TO THIOBENCARB FOR 96 HOURS

	Number Dead (n = 10)			
Concentration (mg/L)	24 Hr	48 Hr	72 Hr	96 Hr
0.000	0	0	0	0
0.007	0	0	0	0
0.056	0	0	0	0
0.137	0	0	0	0
0.675	0	4	7	10
1.220	0	4	10	10
LC50 (mg/L)	>1.220	>1.220	0.440	0.304
95% Confidence Limits			(0.278-0.666).	(0.137-0.675)

The results of the 96-hour exposure to molinate are summarized in Table 2. These data suggest that molinate is less toxic to neomysids than thiobencarb by a factor of about 30, based on a 4-day exposure. However, the pattern of decreasing LC50 values over time again suggests cumulative toxicity.

Table 2

MORTALITY OF NEOMYSIDS EXPOSED TC MOLINATE FOR 96 HOURS

Concentration (mg/L)	Number Dead (n = 10)			
	24 Hr	48 Hr	72 Hr	96 Hr
0.00	0	0	0	0
0.09	0	1	1	1
0.68	0	0	0	0
1.30	0	0	0	0
7.10	0	0	0	0
12.38	0	2	3	8
LC50 (mg/L)	>12.38	>12.38	>12.38	9.91
95% Confidence Limits				(7.65-13.57)

Time-Independent Studies

The results of the time-independent study on thiobencarb are summarized in Table 3. These data indicate that thiobencarb has high toxicity to neomysids, as indicated by the 18-day LC50 estimate of 0.053 mg/L. Although the toxic response appeared to reach a threshold between Days 16 and 18, the steady decrease in LC50 values over time is again indicative of cumulative toxicity. It should also be pointed out that although it appeared that a threshold was reached in terms of continued mortality, all of the concentrations resulted in a toxic response, i.e., 10% and 30% mortality, occurred at 2 and 20 ppb thiobencarb, respectively. Thus, this test did not provide any indication of a no-observable effect level (NOEL).

Table 3

MORTALITY OF NEOMYSIDS EXPOSED TO THIOBENCARB FOR 18 DAYS

Concentration (mg/L)	Number Dead (n = 20)			
	Day 4	Day 7	Day 14	Day 18
0.000	0	0	0	0
0.002	1	1	2	2
0.020	0	1	4	6
0.048	1	1	3	7
0.279	2	12	20	20
0.375	8	20	20	20
LC50 (mg/L)	0.375	0.214	0.091	0.053
95% Confidence interval		(0.174-0.258)	(0.052-0.137)	(0.032-0.100)

The results of the time-independent study on molinate are summarized in Table 4. These data indicate that molinate has appreciable toxicity to neomysids, although not as great as thiobencarb; the threshold LC50 for molinate (0.23 mg/L) was four times higher than that for thiobencarb (0.053 mg/L). As with thiobencarb, molinate showed marked cumulative toxicity, with the LC50 decreasing from 8.30 to 0.23 mg/L over the period of 4 to 28 days of exposure. This test also did not provide experimental evidence of a NOEL; 25% mortality occurred at the lowest treatment level of 0.16 mg/L.

Table 4

MORTALITY OF NEOMYSIDS EXPOSED TO MOLINATE FOR 28 DAYS

Concentration (mg/L)	Number Dead (n = 20)			
	Day 4	Day 7	Day 14	Day 28
0.000	0	0	2	2
0.161	0	0	0	5
0.899	0	0	11	19*
1.587	1	5	17	19*
9.300	14	19	19	19*
16.474	12	20	20	20
LC50 (mg/L)	8.30	2.53	0.82	0.23
95% Confidence Limits	(5.66-12.74)	(1.87-3.56)	(0.57-1.04)	(0.15-0.34)

*100% mortality (1 neomysid leaped from dish during exposure).

The response of the neomysids exposed to a combination of molinate and thiobencarb is summarized in Table 5. These results suggest that the mixture is quite toxic to neomysids but that the presence of the chemicals in combination does not particularly enhance the toxic effect. In addition, the lack of appreciable effect at the lowest concentration suggests that the toxic threshold concentration for the two chemicals lies in the vicinity of 0.004 mg/L for thiobencarb and 0.14 mg/L for molinate.

Table 5

MORTALITY OF NEOMYSIDS EXPOSED TO A COMBINATION OF MOLINATE AND THIOBENCARB FOR 18 DAYS

Thiobencarb	Molinate	Day 4	Day 7	Day 14	Day 18
0	0	0	0	1	2
0.004	0.143	0	0	1	2
0.024	0.783	0	0	9	18
0.054	1.579	5	8	20	20
0.342	8.716	11	20	20	20
0.440	16.704	20	20	20	20
LC50 (mg/L total)		4.80	2.15	0.79	0.30
95% Confidence Limits		(3.34-6.96)	(1.62-2.99)	(0.62-0.98)	(0.21-0.43)
LC50 (mg/L thiobencarb)		0.156	0.071	0.024	0.009
LC50 (mg/L molinate)		4.64	2.08	0.77	0.29

Toxicity Interactions

By calculating the ratio of the actual LC50 estimate for the mixture to the expected LC50 estimate for the mixture, one can infer whether the components of the mixture are synergistic, antagonistic, or merely additive. Table 6 summarizes these ratios for different time periods as well as their associated 95% confidence intervals. Note that the ratios are expressed as logarithmic values to symmetrize their range around zero, as explained under Methods.

Table 6

INTERACTIVE INDICES (S) OF THIOBENCARB AND MOLINATE DURING BIOASSAY OF JOINT TOXICITY TO NEOMYSID SHRIMP

Exposure Period (Days)	Log S	95% Confidence Interval
7	0.056	-0.111-0.223
14	0.055	-0.093-0.203
Final*	0.158	-0.054-0.370

*18 days for thiobencarb and mixture; 28 days for molinate.

The positive values for log S suggest that the two chemicals are somewhat antagonistic in action. However, these values are small and their associated confidence intervals overlap zero. Therefore, we conclude that the antagonistic effect is small, if present at all, and the behavior of the chemicals is additive for all practical purposes.

Summaries of water quality and analytical data associated with these tests are appended.

DISCUSSION

Both molinate and thiobencarb exhibited appreciable toxicity to neomysids. The incipient LC50 values* of 0.053 and 0.23 mg/L for thiobencarb and molinates respectively, also indicate that thiobencarb is significantly more toxic than molinate. Both chemicals exhibited cumulative toxicity; it took 18 and 28 days, respectively, for thiobencarb and molinate to reach toxicity thresholds when the mortality stabilized.

To place the results of these studies in context with environmental exposures, it is appropriate to consider the actual levels of these chemicals in the environment. According to data at the State Water Resources Control Board (Dr. John Cornacchia, personal communication), levels of thiobencarb and molinate reached 2 and 13 µg/L in the upper Sacramento-San Joaquin Delta over a 2- to 3-week period in 1985. For thiobencarb, this value is below the incipient LC50 by a factor of about 25, hence appearing to offer a reasonable degree of protection. It is also below by a factor of 10, a level that resulted in 30% mortality over an 18-day period and it is right at the level that resulted in 10% mortality over the same period. Thus, in the absence of additional stresses, this concentration might be expected to produce minimal acute effects on the population.

Environmental concentrations of molinate (13 µg/L)were below the incipient LC50 by a factor of about 18 and below the level that caused 25% mortality by a factor of 12. Because we did not test concentrations below that which caused 25% mortality, it is difficult to assess the likely level of effect at 13 µg/L.

No discernible increase in mortality was seen in the lowest treatment level in the test series that contained a mixture of thiobencarb and molinate. This suggests that these concentrations--0.004 mg/L thiobencarb and 0.143 mg/L molinate--are near the threshold for toxicity of these chemicals to neomysids. Thus, these data also suggest that environment concentrations of thiobencarb lie at or near the threshold of acute toxicity, whereas environmental concentrations of molinate are lower than the threshold of acute toxicity by a factor of about 10.

Although these data do provide information on how adult neomysids might respond to these chemicals over a 2- to 4-week period, they do not

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*The concentration at which 50% of the test organisms might be expected to survive indefinitely.

provide any information on the response of sensitive life stages or effects on other parameters such as growth. The current data suggest that ambient levels of the herbicides do not pose an appreciable acute toxicity hazard, but additional tests will have to be performed before the potential impacts of chronic toxicity can be judged.

Appendix

WATER QUALITY AND ANALYTICAL CHEMICAL DATA

Table A-1

SUMMARY OF CHEMICAL CONCENTRATIONS ASSOCIATED WITH THE 96-HR ACUTE BIOASSAYS ON NEOMYSIDS

Chemical	Measured Concentrations (mg/L)			
	$\bar{x}$	SD	n	Range
Thiobencarb	0.000	0.000	3	—
	0.007	0.001	3	0.006-0.008
	0.056	0.004	3	0.054-0.060
	0.137	0.004	3	0.133-0.139
	0.675	0.070	4	0.676-0.752
	1.220	0.203	3	1.020-1.424
Molinate	0.000	0.000	3	—
	0.09[illegible]	0.080	3	0.00- 0.142
	0.68[illegible]	0.083	4	0.556- 0.732
	1.29[illegible]	0.158	4	1.065- 1.416
	7.0[illegible]6	0.684	4	6.070- 7.460
	12.375	1.522	4	10.125-13.320

Table A-2

SUMMARY OF CHEMICAL CONCENTRATIONS ASSOCIATED WITH THE TIME-INDEPENDENT BIOASSAYS ON NEOMYSIDS MERCEDIS

Chemical	Measured Concentrations (mg/L) $\bar{x}$	SD	n	Range
Thiobencarb (mixture)	0.000	0.0000	5	--
	0.004	0.0018	5	0.002-0.005
	0.024	0.0086	5	0.014-0.034
	0.054	0.0364	3	0.029-0.096
	0.342	0.1243	3	0.253-0.484
	0.440	0.1280	2	0.349-0.530
Molinate (mixture)	0.000	0.0000	5	--
	0.143	0.0109	5	0.125- 0.153
	0.783	0.0584	5	0.704- 0.860
	1.579	0.2023	3	1.412- 1.804
	8.716	1.1187	3	7.329- 9.456
	16.704	0.7672	2	16.162-17.247
Thiobencarb (alone)	0.000	0.0000	5	--
	0.002	0.0009	5	0.001-0.003
	0.020	0.0116	5	0.010-0.040
	0.048	0.0217	5	0.028-0.082
	0.279	0.0642	4	0.195-0.349
	0.375	0.0441	3	0.333-0.421
Molinate (alone)	0.000	0.0000	8	--
	0.161	0.0120	8	0.153- 0.188
	0.899	0.0954	6	0.814- 1.047
	1.587	0.1595	5	1.373- 1.699
	9.300	1.2100	3	7.923-10.192
	16.474	2.2272	3	14.481-18.878

Table A-3

WATER QUALITY ASSOCIATED WITH THE 96-HR ACUTE BIOASSAYS
ON NEOMYSIS MERCEDIS

Chemical	Treatment Level	Temperature (°C) $\bar{x}$	SD	n	Range	Dissolved Oxygen (mg/L) $\bar{x}$	SD	n	Range	pH $\bar{x}$	SD	n	Range	Conductivity (µmhos) $\bar{x}$	SD	n	Range
Thiobencarb	0	17.5	0.5	5	17.0-18.0	8.6	1.2	2	7.7-9.4	8.1	0.1	2	8.0-8.1	3650	71	2	3600-3700
	1	17.5	0.5	5	17.0-18.0	8.5	1.[illegible]	2	7.6-9.4	8.2	0.1	2	8.1-8.2	3700	0	2	--
	2	17.5	0.5	5	17.0-18.0	8.5	1.[illegible]	2	7.6-9.4	8.2	0.1	2	8.1-8.2	3700	0	2	--
	3	17.5	0.5	5	17.0-18.0	8.6	1.3	2	7.6-9.5	8.2	0.1	2	8.1-8.2	3700	0	2	--
	4	17.5	0.5	5	17.0-18.0	8.5	1.3	2	7.6-9.4	8.2	0.1	2	8.1-8.2	3675	35	2	3650-3700
	5	17.5	0.5	5	17.0-18.0	8.5	1.3	2	7.6-9.4	8.2	0.1	2	8.1-8.2	3725	35	2	3700-3750
Molinate	0	17.5	0.5	5	17.0-18.0	8.7	1.2	2	7.8-9.5	8.2	0.1	2	8.1-8.3	3700	0	2	--
	1	17.5	0.5	5	17.0-18.0	8.6	1.2	2	7.7-9.4	8.2	0.1	2	8.1-8.2	3675	35	2	3650-3700
	2	17.5	0.5	5	17.0-18.0	8.6	1.2	2	7.7-9.4	8.1	0.1	2	8.0-8.2	3675	35	2	3650-3700
	3	17.5	0.5	5	17.0-18.0	8.6	1.2	2	7.7-9.4	8.1	0.1	2	8.0-8.2	3675	35	2	3650-3700
	4	17.5	0.5	5	17.0-18.0	8.5	1.1	2	7.7-9.3	8.2	0.1	2	8.1-8.2	3675	35	2	3650-3700
	5	17.5	0.5	5	17.0-18.0	8.5	1.1	2	7.7-9.3	8.2	0.1	2	8.1-8.2	3675	35	2	3650-3700

Table A-4

WATER QUALITY ASSOCIATED WITH TIME-INDEPENDENT BIOASSAYS ON NEOMYSIS MERCEDIS

Chemical	Treatment Level	Temperature (°C)				Dissolved Oxygen (mg/L)				pH				Conductivity (µmhos)			
		$\bar{x}$	SD	n	range	$\bar{x}$	SD	n	Range	$\bar{x}$	SD	n	Range	$\bar{x}$	SD	n	Range
Thiobencarb	0	17.9	1.4	5	16.0-20.0	8.7	0.4	5	8.3-9.2	8.7	0.1	5	8.6-8.9	3810	22	5	3800-3850
	1	17.9	1.4	5	16.0-20.0	8.7	0.4	5	8.3-9.2	8.7	0.1	5	8.6-8.9	3810	22	5	3800-3850
	2	17.9	1.4	5	16.0-20.0	8.6	0.4	5	8.3-9.2	8.8	0.1	5	8.5-8.9	3800	0	5	3800-3850
	3	17.9	1.4	5	16.0-20.0	8.6	0.4	5	8.2-9.2	8.8	0.1	5	8.6-8.9	3780	45	5	3700-3800
	4	18.4	1.0	4	18.0-20.0	8.7	0.3	4	8.2-9.2	8.8	0.1	4	8.6-8.8	3733	48	4	3700-3800
	5	19.0	1.4	2	18.0-20.0	8.8	0.4	2	8.5-9.0	8.8	0	2	—	3775	35	2	3750-3800
Molinate	0	18.1	1.1	8	16.0-20.0	8.5	0.4	8	9.1-9.2	8.7	0.1	8	8.4-8.8	3800	0	8	—
	1	18.1	1.1	8	16.0-20.0	8.5	0.4	8	8.1-9.2	8.7	0.2	8	8.3-8.8	3800	0	8	—
	2	17.9	1.3	6	16.0-20.0	8.5	0.3	6	8.1-9.2	8.7	0.1	6	8.5-8.8	3800	0	8	—
	3	17.9	1.4	5	16.0-20.0	8.7	0.4	5	8.2-9.2	8.8	0.1	5	8.7-8.8	3780	45	5	3700-3800
	4	19.0	1.4	2	18.0-20.0	8.9	0.5	2	8.5-9.2	8.8	0.1	2	8.7-8.8	3775	35	2	3750-3800
	5	19.0	1.4	2	18.0-20.0	8.7	0.4	2	8.4-8.9	8.0	0	2	—	3675	35	2	3650-3700
Mixture	0	17.9	1.4	5	16.0-20.0	8.8	0.3	5	8.3-9.2	8.8	0.1	5	8.7-8.8	3800	0	5	—
	1	17.9	1.4	5	16.0-20.0	8.8	0.3	5	8.3-9.2	8.8	0.1	5	8.7-8.8	3800	0	5	—
	2	17.9	1.4	5	16.0-20.0	8.7	0.4	5	8.3-9.2	8.8	0.1	5	8.7-8.8	3800	0	5	—
	3	18.5	1.3	3	17.5-20.0	8.7	0.5	3	8.3-9.2	8.8	0	3	—	3800	0	3	—
	4	19.0	1.4	2	18.0-20.0	8.9	0.5	2	8.5-9.2	8.8	0	2	—	3750	71	2	3700-3800
	5	20.0	0	1	—	9.2	0	1	—	8.8	0	1	—	3600	0	1	—

APPENDIX F-3

SRI International

CHRONIC TOXICITY OF RICE HERBICIDES TO NEOMYSIS MERCEDIS

Final Report

29 November 1985

Prepared for:

CALIFORNIA STATE WATER RESOURCES CONTROL BOARD
901 P Street, Third Floor
Sacramento, California 95814

Attention: Dr. John Cornacchia

Submitted by:

Howard C. Bailey, Director
Aquatic Toxicology Program

SRI Project LSU-7578

Approved:

Jon B. Reid, Director
Toxicology Laboratory

W. A. Skinner, Vice President
Life Sciences Division

SRI International®
333 Ravenswood Ave. • Menlo Park, CA 94025
415 326-6200 • TWX: 910-373-2046 • Telex: 334-486

QUALITY ASSURANCE UNIT

Final Report Statement

SRI International assures the quality and integrity of this study, Chronic Toxicity Of Rice Herbicides To Neomysis Mercedis, for the California State Water Resources Control Board.

The study was inspected on August 2, 1985 during the termination phase. The findings of the Quality Assurance Unit inspection were reported at the time of the inspection to the Study Director. SRI management was informed of the inspection results on August 3, 1985. A data audit was performed on November 8, 1985. The Study Director and SRI management were informed of the audit results on November 11, 1985.

The final report was audited and reviewed on November 22, 1985. The results of the final report review were communicated to the Study Director and SRI management on November 25, 1985. The final report accurately describes the methods and standard operating procedures and reflects the raw data of the study. Any deviations from the approved protocol and standard operating procedures were made with proper authorization and documentation.

Manager of Regulatory Affairs
and Quality Assurance

11/25/85
Date

CONTENTS

INTRODUCTION

At the request of the California State Water Resources Control Board, SRI International performed a flow-through chronic toxicity studies on rice herbicides, using Neomysis mercedis as the test organism. The objective of the study was to provide data on the chronic toxicity of thiobencarb, molinate, and a mixture of the two. Testing was initiated on 27 March 1984 and completed on 2 August 1985.

Copies of this Final Report and the laboratory notebook containing the raw data for this study will be retained in Building 253, Room C-2, for one year from the date of the Final Report. Thereafter, these materials will be stored in SRI's Records Center.

The Study Director on this project was Howard C. Bailey, who was assisted by Paul A. Haskins, Kevin Joe, Jesse Martin, and Anne Tait, biological technicians.

MATERIALS AND METHODS

Test Chemicals

The test chemicals were technical-grade molinate and thiobencarb. Molinate was obtained from Stauffer Chemical Company and thiobencarb was obtained from Chevron Chemical Company. The purity and stability of the materials was not verified by SRI. The test chemicals were stored in a hood at room temperature at SRI.

Test Organism

The test organisms were neomysid shrimp (Neomysis mercedis). They were obtained from cultures maintained at SRI and originating from stock collected in Montezuma Slough in December 1982. The cultures were housed in 15-gallon glass aquaria containing reconstituted water at 3000 to 4000 µmhos and were fed Artemia nauplii. All neomysids appeared to be healthy and feeding well.

Diluent Water

Diluent water was prepared by mixing dechlorinated tap water and artificial seawater (Instant Ocean) to achieve a final conductivity of approximately 3500 µmhos. This water was made in 50-gallon batches and stored at 16 to 19°C in Nalgene containers until use.

Test Procedures

Neomysids were exposed to nominal concentrations of 0.00, 3.3, 6.6, 13.3, 26.5, and 53.0 µg/L thiobencarb and 0.00, 14.6, 29.1, 58.3, 116.5, and 233.0 µg/L molinate. Additional neomysids were exposed to these concentrations of each chemical together as a mixture to assess the potential for interactive effects on chronic toxicity. These concentrations were selected on the basis of time-independent acute toxicity studies conducted previously. The concentrations were made by mixing appropriate volumes of

diluent water and aqueous solutions of the test chemicals in Mariotte bottles, which delivered the test solution to two replicate 150 mm × 75 mm crystallizing dishes for each concentration. Each dish received a minimum of two dish volumes of solution per day. These dishes drained into a second tier of dishes, which drained into glass catch bottles. Test temperature was maintained between 16 and 18°C and a 16-hr L:8-hr D photoperiod was used.

The test was initiated by selecting gravid females from the different culture tanks and randomly distributing them individually to small culture dishes containing a mixture of culture and diluent water. This procedure was continued until all of the small dishes contained three gravid females. Randomly selected dishes were then drained gently into the exposure dishes, resulting in three females per dish, with some females left over to be used in case of incidental deaths. If an incidental death occurred during the first seven days of the test, the female was replaced.

The primary purpose of this procedure was to ensure sufficient numbers of young of approximately the same age at each treatment level. The dishes were inspected daily; any young produced were noted and then transferred via pipet to rearing dishes (150 mm × 75 mm) that received inflowing water from the drain tube of the dish in which they were hatched.

After 14 days, the surviving adults were removed from the upper tier of dishes. The young were then pooled and distributed to the upper dishes so that each dish received 15 to 20 young. If there were not enough young to provide 15 for each dish, replicate A was filled first, then replicate B.

Each dish was inspected daily and any dead young were counted and removed. On Day 42, the dishes were photographed over millimeter grid paper so that the sizes of the neomysids could be determined and the test terminated.

Neomysids on test were fed brine shrimp nauplii daily. Uneaten nauplii were removed daily with a 50- or 100-ml syringe. In addition, an algal and vitamin supplement was provided three times per week.

The first tests on molinate and thiobencarb had to be terminated after 14 days because failure of the bioassay room's temperature-control system resulted in excessive deaths due to unacceptably high temperatures. New tests were begun at a later date when sufficient numbers of gravid females became available. The first test on the mixture was continued primarily because it was affected least by the increase in temperatures; in the dishes where deaths did occur, the losses were replenished by survivors from the groups exposed to the single chemicals at the same concentration. Thus, the results from exposure to the mixture may reflect temperature-induced stress as well as direct chemical toxicity.

Water-Quality Measurements

Temperature was measured daily in the test solutions at each concentration and in the controls. Dissolved oxygen, conductivity, and pH in each concentration were determined weekly. The instruments used were manufactured by Yellow Springs Instrument Co. (dissolved oxygen and conductivity meters), Orion Ion Analyzer (pH meter), and Scientific Products (thermometer).

Chemical Analyses

Samples were taken from the test solutions twice weekly. The two samples from each concentration were pooled before analysis. Samples were placed in amber bottles with Teflon-lined caps and refrigerated until extraction and analysis. The analytical procedures follow.

An aliquot of thiobencarb, ranging in volume from 100 to 750 ml, was decanted into an appropriately sized separatory funnel and extracted with two portions of dichloromethane (DCM). The volume of DCM depended on the volume of sample used. It was found that a 1:8 ratio (DCM:sample) worked best. For example, if 400 ml of sample was to be extracted, then two

50-ml portions of DCM were used in sequence. A 1.0-ml portion of iso-octane (2,2,4-trimethylpentane) was added to the combined extracts as a keeper. This solution was concentrated to a volume of approximately 5 ml, using a rotary evaporator at a bath temperature not to exceed 35°C. This concentrated extract was transferred to an 8-ml test tube; the rotary evaporation flask was rinsed twice with DCM, bringing the volume to approximately 8 ml. This solution was further concentrated, under nitrogen gas, to a final volume of 1.0 ml. Enough internal standard, 3-amino-2,4-DNT (in DCM), was added so that "mg on column" values for 3-amino-2,4-DNT and herbicide were roughly equivalent.

A 200-ml portion of molinate was decanted into a 250-ml separatory funnel and extracted twice with 25-ml portions of DCM. The extracts were pooled and an appropriate amount of 3-amino-2,4-DNT was added as internal standard.

All samples were analyzed using gas chromatography (Varian Model 3700) with TSD detection (nitrogen-phosphorous specific). An HP3390 integrator was used along with a Supercoport SP2250, 100/120, column. Injection volumes ranged from 1 to 5 µl at 205°C isothermal. Gas flow rates were: air, 175 ml/min; H_2, 30 ml/min; and N_2, 30 ml/min.

Statistical Analyses

Means and standard deviations were calculated for the growth and water-quality data as well as for the chemical measurements.

Survival curves were computed by the Kaplan-Meir product-limit method using the computer program BMDP1L (1). Survival times were computed from the time of transfer of the shrimp. Leapers and surviving shrimp were treated as censored observations. If the exact time of death/leaping was not determinable, the event was assigned to the middle day of the week in which the event happened.

Mean survival times were computed from the fitted curves. In cases where there were surviving shrimp, the means are mean survival during the

period of the study and are somewhat less than the total mean survival, which cannot be estimated from these data.

Differences between survival curves were tested with two nonparametric tests: the Mantel-Cox test (a generalized Savage test) and the Breslow test (a generalized Wilcoxon test) (2,3). The two tests differ in how the observations are weighted; the Breslow gives more weight to the earlier events and the Mantel-Cox gives more weight to later events in the comparison.

Dunnett's test for multiple comparisons in the analysis of variance of several treatments versus a control was applied to test the effect of concentration on the number of adult females remaining at the end of the hatching period and the number of young at birth, posthatch, and at transfer (4). Two-sided tests at level $p = 0.05$ were computed. To stabilize the variance in the number of young, tests were also computed on the natural logarithms of the number of young (to avoid problems with zeros, 0.5 was added to each count before taking logarithms).

Dunnett's test was also applied to the proportion of posthatch young to young born and the proportion of young at transfer to young at posthatch. (To avoid problems with zeros, 0.5 was added to the numerator and 1 was added to the denominator of each ratio.) To stabilize the variance, tests were also computed on the logit transforms of the proportions by

$$\text{logit}(p) = \log[p/(1-p)] \quad .$$

For both of these tests, weighted analyses of variance were computed, with a weight equal to the denominator of the ratio.

RESULTS

The F_0 reproduction data for neomysids exposed to thiobencarb and molinate is summarized in Table 1. There appeared to be no significant concentration-related effects on young produced or on survival of young during the first 14 days of exposure for either of the two chemicals. However, survival of young was less than the control values in most of the treatment groups by Day 14. The number of adult females surviving until the end of the first 14 days reflects the stress during the critical period of expulsion of the brood and the subsequent moult. It does not appear that there was an appreciable chemical-related effect on adult female survival for either of the two chemicals.

Table 1

REPRODUCTIVE DATA FOR NEOMYSIDS EXPOSED TO RICE HERBICIDE

Concentration (µg/L)	Avg. No. Young Produced	Avg. No. Young Surviving at 14 Days	Avg. No. Adult Surviving Females at Day 14
Thiobencarb			
Control	29	20	2.5
3.2	19.5	12	2.5
6.2	16.0	5	2.0
12.8	25.0	13.5	2.5
23.5	23.0	7.5	1.5
53.4	25.5	13.5	1.5
Molinate			
Control	33	17.5	2.5
15.1	40	17.5	3.0
25.6	26	15.0	2.5
45.2	6.5[a]	1.5	1.0
89.6	22.5	12.0	2.5
173.7	19.0	3.5	1.0

[a]Significantly less than the control value ($p \leq 0.05$).

The growth of the juvenile neomysids after 42 days of exposure to rice herbicides is summarized in Table 2. For thiobencarb, growth was significantly reduced at 12.8 and 53.4 µg/L; there were no survivors at 23.5 µg/L. Growth was not affected at 3.2 and 6.2 µg/L. For molinate, length was significantly reduced at 89.6 µg/L but not at 15.1 or 25.6 µg/L; there were no survivors at 45.2 µg/L. The largest neomysid occurred in the highest concentration, 173.7 µg/L, but this was probably a density-dependent artifact--only one neomysid was left in this concentration. In the mixture of thiobencarb and molinate, growth was significantly reduced at 142.9 µg/L but not at 34.8 or 66.3 µg/L; there were no survivors at the highest concentration, 268 µg/L.

The mean survival times of neomysids reared in rice herbicides are presented in Table 3. The survival curves are shown in Figures 1, 2, and 3, respectively, for the thiobencarb, molinate, and mixture exposures. For thiobencarb, concentrations of 12.8 to 53.4 µg/L resulted in significantly reduced survival time compared with the control value. The reduction in survival at 6.2 µg/L was nearly significant ($p \approx 0.06$) and since inspection of the survival curve (Figure 1) for this concentration shows that it is different from the curves for the control and 3.2 µg/L, we would also consider survival reduced at 6.2 µg/L.

The results for molinate were clear-cut. Survival was significantly reduced at concentrations of 45.2 to 173.7 µg/L but not in 15.1 or 25.6 µg/L. The results obtained for the mixture were interesting in that survival was reduced in all of the test concentrations--17.2 to 268 µg/L--compared with the control. This could reflect a synergistic effect. Because there was only a slight effect on survival time at the second lowest concentration of thiobencarb (6.2 µg/L) and no effect at the second lowest concentration of molinate (25.6 µg/L) we might expect that the second lowest concentration of the mixture, 34.8 µg/L, which contained approximately the same concentrations of thiobencarb and molinate as those used in the tests on the individual substances, would also produce

Table 2

AVERAGE LENGTHS OF N. MERCEDIS AFTER 42 DAYS OF EXPOSURE TO RICE HERBICIDES

Concentration (µg/L)	Length (cm) $\bar{x}$	SD	n
Thiobencarb			
Control	0.82	0.18	33
3.2	0.74	0.15	19
6.2	0.86	0.15	7
12.8	0.70*	0.13	12
23.5	--	--	--
53.4	0.55*	0.07	2
Molinate			
Control	0.87	0.19	21
15.1	0.75	0.15	23
25.6	0.82	0.23	24
45.2	--	--	--
89.6	0.60*	0.11	8
173.7	1.00	0.00	1
Mixture			
Control	1.00	0.23	9
17.2	--	--	--
34.8	1.00	0.00	2
66.3	0.95	0.10	4
142.9	0.65[a]	0.06	4
268.0	--	--	--

[a]Significantly different from the control; $p \leq 0.05$.

Table 3

MEAN SURVIVAL TIME FOR NEOMYSIDS REARED IN SOLUTIONS OF RICE HERBICIDES

Concentration (µg/L)	Mean Survival Time (Days)
Thiobencarb	
Control	30.8
3.2	29.3
6.2	25.0[b]
12.8	20.5[a]
23.5	2.1[a]
53.4	8.4[a]
Molinate	
Control	29.6
15.1	28.7
25.6	30.6
45.2	10.3[a]
89.6	17.8[a]
173.7	6.0[a]
Mixture	
Control	19.9
17.2	6.8[a]
34.8	10.4[a]
66.3	14.2[b]
142.9	11.8[a]
268.0	9.8[a]

[a]Significantly less than the control; $p \leq 0.05$.

[b]Significantly less than the control; $0.05 < p < 0.07$.

CUMULATIVE PROPORTION SURVIVING A = Control; B = 3.2 µg/L; C = 6.2 µg/L;

D = 12.8 µg/L; E = 23.5 µg/L; F = 53.4 µg/L

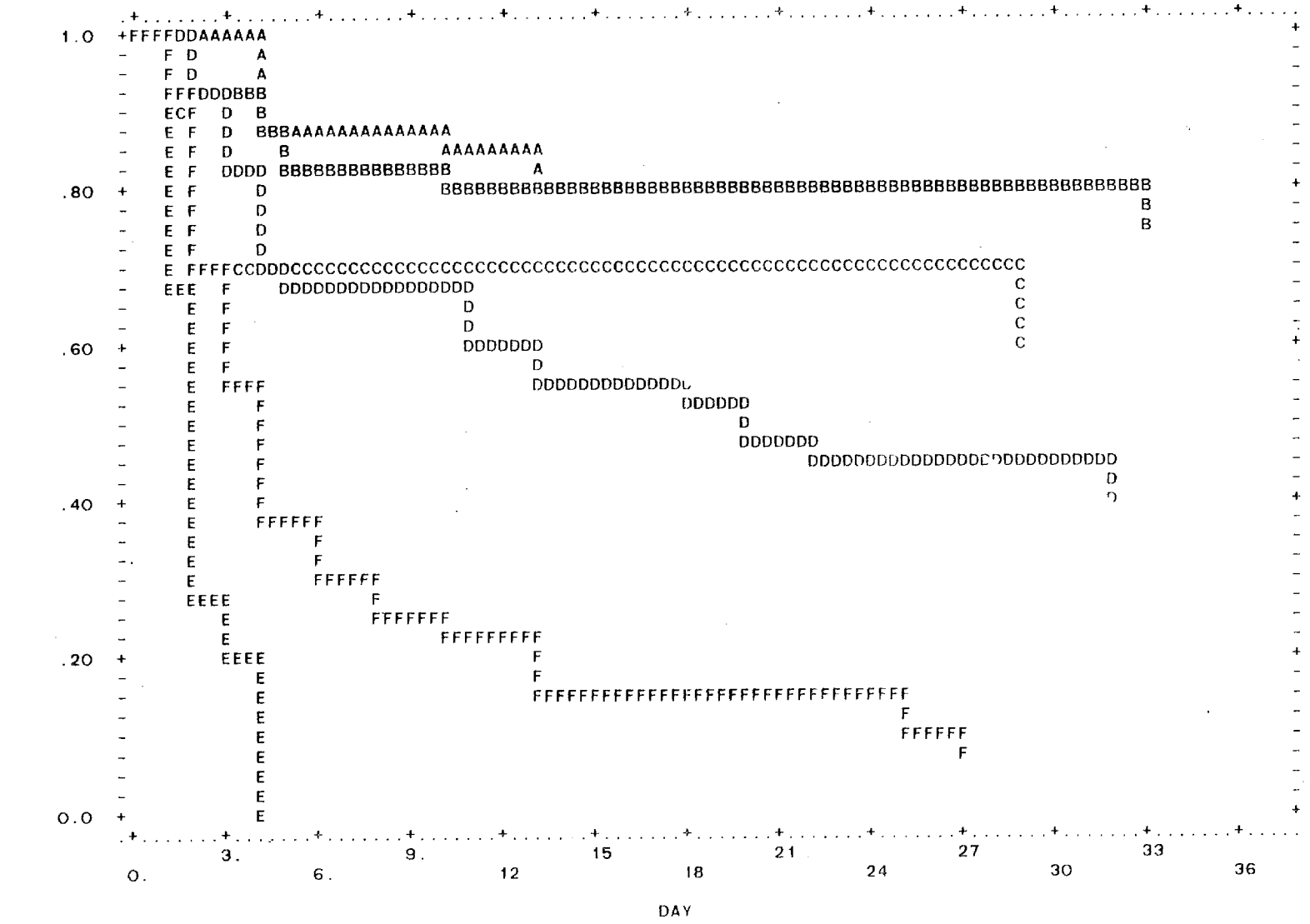

Figure 1. Survival Curves for N. mercedis Exposed to Thiobencarb for 42 Days.

CUMULATIVE PROPORTION SURVIVING A = Control; B = 15.1 µg/L; C = 25.6 µg/L;

D = 45.2 µg/L; E = 89.6 µg/L; F = 173.7 µg/L

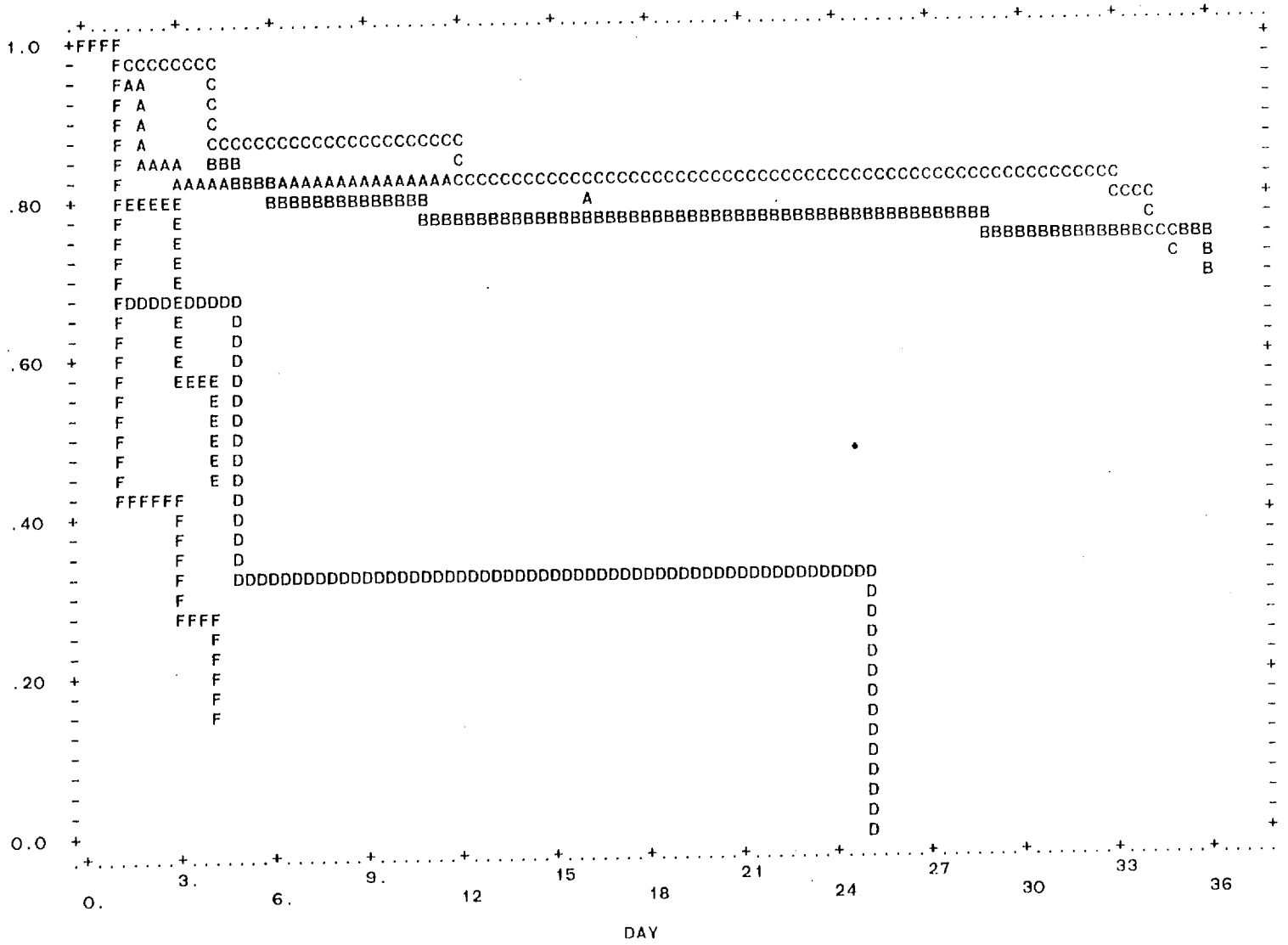

Figure 2. Survival Curves for N. mercedis Exposed to Molinate for 42 Days.

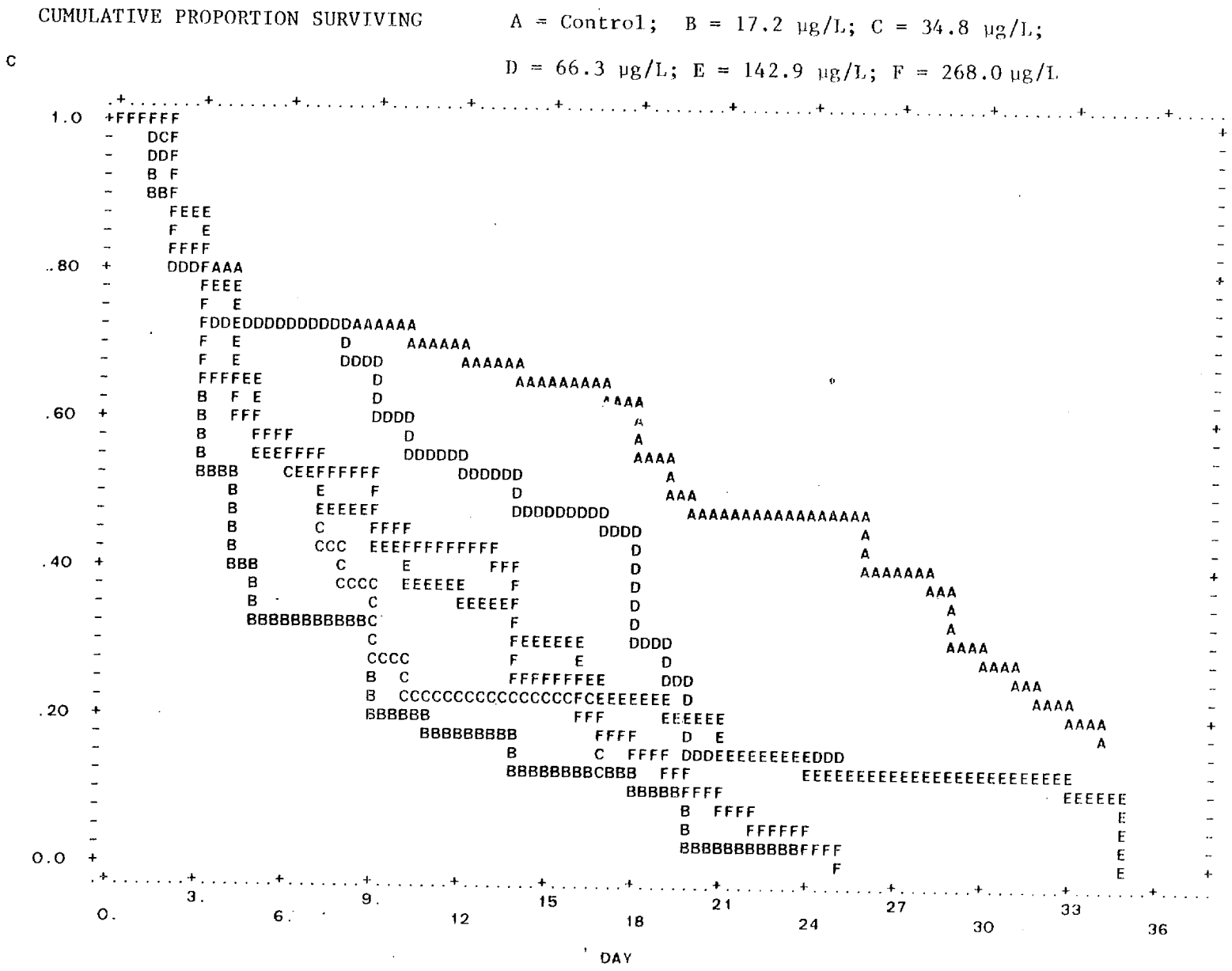

Figure 3. Survival Curves for N. mercedis Exposed to a Mixture of Thiobencarb and Molinate for 42 Days.

an effect. Along the same line of reasoning, the fact that the lowest concentration of the mixture, 17 µg/L, produced a significant effect could be attributed to toxicity interactions since the lowest treatment levels of thiobencarb (3.2 µg/L) and molinate (15.1 µg/L) failed to produce any measurable adverse effects. Such an interaction would be plausible since earlier work (5,6) demonstrated that molinate and thiobencarb are additive in effect when tested together. However, at least two other hypotheses could be responsible for the observed effects of low levels of the mixture. First, the test on the mixture was initiated earlier than the individual tests on the two chemicals, so differences in sensitivity attributable to differences in the test organisms could explain the sensitive response to the mixture. Second, as described earlier, the organisms exposed to the mixture were under thermal stress during the early part of the study, which may have impaired their ability to tolerate the test chemicals. Supporting evidence for either genetic differences or the effect of stress in the mixture experiment can be obtained by looking at the survival data for the control groups. Survival time of controls averaged 30 days in the tests on the individual chemicals and only 20 days in the test on the mixture. This implies fundamental differences between the two test series and suggests that caution should be exercised when comparing the results.

Data on water quality and chemical analysis for these studies are appended.

DISCUSSION

The results of the chronic tests on individual chemicals indicate that the "no-effect" level lies between 3.2 and 6.2 µg/L for thiobencarb and between 25.6 and 45.2 µg/L for molinate. In both cases, the mean survival time was the most sensitive parameter tested.

To place the results of these studies in context with environmetnal exposures, it is appropriate to consider the actual levels of these chemicals in the environment. According to data at the State Water Resources Control Board (Dr. John Cornacchia, personal communication), levels of thiobencarb and molinate reached 3 and 13 µg/L, respectively, in the upper Sacramento-San Joaquin Delta over a 2- to 3-week period in 1985. For thiobencarb, this value is below the no-effect level of 3.2 µg/L by a factor of 1.6 and below the lowest effective concentration of 6.2 µg/L by a factor of 3.2. The environmental concentration of molinate (13 µg/L) was less than the no-effect level of 25.6 µg/L by a factor of 2 and below the lowest effective concentration (45.2 µg/L) by a factor of 3.5 Thus, it is apparent that environmental concentrations reach levels only a factor of 2 to 3 less than that which cause effects under laboratory conditions. Whether this might be an adequate margin of safety depends on a number of variables. First, the relatively short exposure time of 2 to 3 weeks in the Delta suggests that these chemicals are not present at high enough concentrations long enough to pose a chronic toxicity hazard. However, there is some uncertainty associated with the no-effect levels derived in this study because the studies do not address any effects on reproduction in the F_1 generation--a potentially important factor. In addition, the effects of stress on toxicity and the synergistic effect when the two chemicals are present together should also be considered, based on the results from the exposure to the mixture.

If we are conservative and assume that the theoretical lowest effective concentrations for the two chemicals individually are the geometric means of their respective "effect-no effect" concentrations, we obtain values of 4.45 µg/L and 34.0 µg/L for thiobencarb and molinate, respectively. Each of these values can be assigned a Toxic Unit value (TU) of 1, indicating that these are the lowest "unit of toxicity" that will result in chronic effects to neomysids under our test conditions. Since the interactive toxicity appears to be additive in nature, we can then look at the levels of the individual chemicals in the lowest concentration of the mixture (see Table A-1 in the appendix) to see if we would expect to find any toxicity. The concentration of thiobencarb was 4.91 µg/L (a TU of 1.10) and the concentration of molinate was 12.3 µg/L (a TU of 0.4), for a total TU of 1.5. Since this value exceeds 1, we would expect the lowest concentration of the mixture to have had a measurable effect even in the absence of thermal stress. By applying the same analysis to the environmental levels of thiobencarb and molinate, we calculate a TU of 0.45 + 0.38 = 0.83. This is very close to a value of 1, which suggests that only a minimal safety factor exists when the two chemicals are present together.

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Appendix

WATER QUALITY AND ANALYTICAL DATA

Table A-1

MEASURED CHEMICAL CONCENTRATIONS ASSOCIATED
WITH CHRONIC STUDIES ON RICE HERBICIDES WITH NEOMYSIS MERCEDIS

Chemical	Treatment Level	$\bar{x}$	S.D.	n	Range	
Thiobencarb alone	0	0.0	0.0	8	--	--
	1	3.2	0.28	9	2.72	3.53
	2	6.2	0.66	8	5.09	6.79
	3	12.8	1.01	8	11.19	13.99
	4	23.5	2.30	3	21.23	25.83
	5	53.4	5.84	8	45.87	63.32
Molinate alone	0	0.0	0.0	8	--	--
	1	15.1	1.48	8	13.38	17.20
	2	24.6	2.53	7	22.15	28.81
	3	45.2	8.06	6	36.30	58.47
	4	89.6	15.89	8	72.08	109.86
	5	173.7	36.41	7	140.26	229.76
Thiobencarb in mixture	0	0.0	0.0	8	--	--
	1	4.91	0.46	5	4.38	5.63
	2	7.80	0.52	8	7.11	8.50
	3	16.12	1.05	8	13.79	17.29
	4	35.51	1.93	6	31.75	37.10
	5	61.53	0.68	5	60.63	62.04
Molinate in mixture	0	0.0	0.0	8	--	--
	1	12.27	1.60	5	10.36	14.44
	2	27.02	4.80	8	20.59	33.12
	3	50.20	7.39	8	39.40	57.84
	4	107.37	8.00	6	99.53	120.13
	5	206.52	35.65	5	164.02	237.30

Table A-2

WATER QUALITY DATA ASSOCIATED WITH CHRONIC STUDIES ON RICE HERBICIDES

Chemical	Treatment Level	Temperature (°C)					Dissolved Oxygen (mg/L)					pH					Conductivity (µmhos)				
		$\bar{x}$	S.D.	n	Range		$\bar{x}$	S.D.	n	Range		$\bar{x}$	S.D.	n	Range		$\bar{x}$	S.D.	n	Range	
Thiobencarb	0	18.0	0.3	48	17.5	18.5	8.8	0.41	14	8.4	9.4	7.2	0.2	14	6.9	7.8	3493	47.5	14	3400	3600
	1	18.0	0.3	48	17.5	18.5	8.8	0.37	14	8.5	9.4	7.3	0.2	14	7.0	7.9	3500	39.2	14	3400	3600
	2	18.0	0.3	48	17.5	18.5	8.8	0.32	14	8.3	9.4	7.4	0.2	14	7.0	7.9	3500	51.9	14	3400	3550
	3	18.0	0.3	48	17.5	18.5	8.8	0.34	14	8.4	9.4	7.4	0.3	14	7.0	7.9	3514	53.5	14	3400	3600
	4	17.9	0.3	18	17.5	18.5	9.0	0.29	7	8.6	9.4	7.5	0.2	7	7.2	7.8	3557	127.2	7	3400	3800
	5	18.0	0.3	48	17.5	18.5	8.8	0.31	14	8.5	9.3	7.5	0.2	14	7.3	7.9	3536	115.1	14	3400	3900
Molinate	0	18.0	0.3	48	17.5	18.5	8.7	0.37	14	8.4	9.2	7.2	0.2	14	7.0	7.6	3500	0	14	3500	3500
	1	18.0	0.3	48	17.5	18.5	8.6	0.36	14	8.0	9.2	7.3	0.2	14	7.1	7.6	3507	26.7	14	3500	3600
	2	18.0	0.3	48	17.5	18.5	8.7	0.41	14	8.0	9.2	7.3	0.2	14	7.1	7.6	3514	36.3	14	3500	3600
	3	18.0	0.4	35	17.5	18.5	8.8	0.41	11	7.9	9.4	7.5	0.1	11	7.2	7.6	3536	80.9	11	3500	3700
	4	18.0	0.4	43	17.5	18.5	8.7	0.31	14	8.3	9.2	7.5	0.1	14	7.2	7.7	3571	91.4	14	3500	3800
	5	18.0	0.3	45	17.5	18.5	8.7	0.31	12	8.3	9.2	7.4	0.2	12	7.1	7.7	3600	112.8	12	3500	3900
Mixture	0	16.9	0.8	48	15.5	19.0	8.6	0.67	19	7.5	9.6	7.5	0.4	19	7.0	8.2	3554	128.4	19	3400	3750
	1	16.7	0.9	25	15.5	19.0	8.7	0.85	10	7.4	9.7	7.8	0.3	10	7.4	8.1	3550	126.9	10	3400	3700
	2	16.9	0.8	43	15.5	19.0	8.7	0.58	15	7.8	9.6	7.7	0.3	15	7.2	8.1	3561	122.9	15	3400	3750
	3	16.9	0.8	45	15.5	19.0	8.5	0.66	15	7.4	9.4	7.6	0.3	15	7.4	8.0	3551	120.0	15	3400	3750
	4	16.9	0.9	36	15.5	19.0	8.6	0.64	13	7.4	9.6	7.7	0.2	13	7.4	8.0	3581	133.1	13	3400	3800
	5	16.7	0.9	25	15.5	19.0	8.5	0.66	10	7.4	8.9	7.8	0.1	10	7.6	7.9	3575	133.9	10	3400	3700

APPENDIX F-4

SRI International

ACUTE TOXICITY OF RICE-FIELD HERBICIDES TO WHITE STURGEON (ACIPENSER TRANSMONTANUS)

Final Report

SRI Project LSU-7578

15 November 1985

Prepared for:

CALIFORNIA STATE WATER RESOURCES CONTROL BOARD
301 P Street, Third Floor
Sacramento, California 95814

Attention: Dr. John Cornacchia

Prepared by:

Howard C. Bailey

Howard C. Bailey, Director
Aquatic Toxicology Program

Approved by:

Jon B. Reid/LTJ

Jon B. Reid, Director
Toxicology Laboratory

W. A. Skinner

W. A. Skinner, Vice President
Life Sciences Division

SRI International®
333 Ravenswood Ave. • Menlo Park, CA 94025
415 326-6200 • TWX: 910-373-2046 • Telex: 334-486

QUALITY ASSURANCE UNIT

Final Report Statement

SRI International assures the quality and integrity of this study, Acute Toxicity Of Rice Field Herbicides To White Sturgeon, for the California State Water Resources Control Board.

The study was inspected on August 2, 1985 during the termination phase. The findings of the Quality Assurance Unit inspection were reported at the time of the inspection to the Study Director. SRI management was informed of the inspection results on August 3, 1985. A data audit was performed on November 8, 1985. The Study Director and SRI management were informed of the audit results on November 11, 1985.

The final report was audited and reviewed on November 8, 1985. The results of the final report review were communicated to the Study Director and SRI management on November 12, 1985. The final report accurately describes the methods and standard operating procedures and reflects the raw data of the study. Any deviations from the approved protocol and standard operating procedures were made with proper authorization and documentation.

Judy Ritter

Manager of Regulatory Affairs
and Quality Assurance

11/12/85

Date

CONTENTS

Introduction

Acute toxicity studies were performed on rice field herbicides thiobencarb and molinate using white sturgeon as the test organism. Exposure periods of 96 hr and 21-22 days were used to determine the sturgeon's tolerance to short- and intermediate-term exposures. In addition, a 22-day study was performed on a mixture of the two chemicals to determine the extent of any toxic interactions. All tests were performed under continuous-flow conditions. Testing was initiated on 24 April 1985 and completed on 28 August 1985.

Copies of this Final Report and the laboratory notebook containing the raw data for this study will be retained in Building 253, Room C-2, for one year from the date of the Final Report. Thereafter, these materials will be stored in SRI's Records Center.

The Study Director on this project was Howard C. Bailey. He was assisted by Paul A. Haskins, Kevin Joe, Jesse Martin, and Anne Tait, biological technicians.

Materials and Methods

Test Chemicals

Thiobencarb and molinate were supplied to SRI via arrangement with the Sponsor. Both materials were specified as Technical Grade, but SRI did not verify their purities. The samples were stored at room temperature in a hood in covered containers.

Test Organisms

White sturgeon used in the 96-hr acute studies were obtained from the University of California, Davis, as 5-day-old fry. These fish were placed on test at 28 days of age after a 23-day acclimation period. Sturgeon used in the time-independent studies were obtained from excess stocks held by Dr. J. Miller of Stauffer Chemical Company. These fish were 75 days old and were placed on test after a two-day acclimation period. During acclimation, fish were held in the diluent water at the test temperature under flow-through conditions. They were fed a combination of Bio-diet,

live tubefex worms, and frozen brine shrimp several times daily. All fish used in the tests appeared to be healthy and were feeding well. Fish used in the 96-hr bioassays averaged 3.7 cm in length (range of 3.1-4.8 cm) and 0.71 g in weight (range of 0.63-0.80 g). Fish used in the time-independent studies averaged 8.1 cm in length (range of 6.5-10 cm) and weighed 2.13 g (range of 1.0-3.6 g).

Diluent Water

Dechlorinated tap water, a soft water that originates in Hetch Hetchy Reservoir in the Sierra Nevada, was used as the diluent water. SRI has cultured a variety of fish and invertebrates in this water and has always found it to be of excellent quality. The composition of the water is presented in Table 1. Hardness and alkalinity of the diluent water was 32 and 34 mg/L (as $CaCO_3$) in the 96-hr studies and 22 and 23 mg/L, respectively, in the time-independent studies.

Test Procedures

Stock solutions were prepared by injecting appropriate volumes of the test chemicals into 3.5 L of diluent water contained in 4-L amber glass bottles while mixing rapidy on a magnetic stirring plate. Nominal stock concentrations were 30 mg/L for thiobencarb and 300 mg/L for molinate. All bottles were mixed for at least 24 hr prior to use, and actual concentrations were determined analytically before dilutions were prepared. Concentrations used in the 96-hr tests were selected on the basis of preliminary range-finding tests. Concentrations used in the time-independent tests were selected on the basis of the results of the 96-hr exposures.

Test concentrations were prepared by mixing the appropriate amount of stock solution and diluent water in 19-L glass Mariotte bottles. Each Mariotte bottle delivered test solution to two replicate exposure vessels for each concentration. Crystallizing dishes (190 × 100 mm) were used for the 96-hr tests and 19-L aquaria were used for the time-independent tests. Flows were set to provide a minimum of two vessel volumes of solution per day.

Table 1

MINERAL CONTENT AND PHYSICAL PROPERTIES OF WATER
FROM CYSTAL SPRINGS RESERVOIR
(Courtesy of the San Francisco Water Department)

Cations (ppm)	
Aluminum	0.05
Arsenic	< 0.01
Barium	< 0.25
Boron	0.07
Cadmium	< 0.002
Calcium	11.6
Chromium	< 0.005
Copper	0.01
Iron	0.09
Lead	< 0.02
Magnesium	4.1
Manganese	< 0.005
Mercury	< 0.0005
Potassium	0.8
Selenium	< 0.0025
Silver	< 0.005
Sodium	6.3
Zinc	0.01
Anions (ppm)	
Bicarbonate	45.5
Carbonate	0.0
Chloride	11.1
Fluoride	0.09
Hydroxide	0.0
Nitrate	0.2
Nitrite	< 0.003
Phosphate	< 0.01
Sulfate	9.4
Nonionics (ppm)	
Total Apparent ABS	< 0.05
Free Ammonia (NH_3)	< 0.05
Dissolved Oxygen (O_2)	8.7
Silica (SiO_2)	6.0
Derived Values (ppm)	
Hardness as $CaCO_2$	46.1
Alkalinity as $CaCO_2$	37.3
Total Residual @ 103°C-105°C	77
Physical Measurements	
Conductivity (µmhos)	136
pH	7.6
Turbidity (units)	1.5
Colors (units)	0

The tests were initiated by removing sturgeon fry from the acclimation tanks and distributing them, two or hree at a time, using stratified random assortment, to the test containers. Each container received ten fry. They were observed daily, and any dead fish were counted and removed. Fish were also fed daily and uneaten food and waste materials were siphoned out before adding the fresh food. Test temperatures were nominally 15 to 16°C in the 96-hr tests and 18 to 19°C in the 14-day tests. These temperatures were determined by the temperatures at which the fry were being held before SRI acquired them. A 16 hr light:8 hr dark photoperiod was used in all tests.

One problem occurred in the time-independent study on thiobencarb in that none of the fish died during the first 10 days of exposure. Analysis of the test solution at the highest concentration indicated that the level of thiobencarb was lower, by a factor of four, than the concentration that resulted in acute effects in the 96-hr exposure. This was verified by subsequent analyses. Consequently, on Day 16 we increased the toxicant delivery flows in the two lowest concentrations to bring these concentrations up to levels that would be likely to produce effects. This did not result in the loss of any information because no dose-related deaths occurred in any of the test aquaria throughout the first 16 days of exposure, so there were still three concentrations with no apparent effects on survival or behavior after 16 days of exposure. The test was continued for a total of 37 days, which correspondes to 21 days of exposure to the modified dose regimen.

Water Quality Measurements

In the 96-hr studies, temperature and dissolved oxygen were measured daily in each treatment group, alternating between replicates. pH and conductivity were determined at the beginning and end of the exposure period. In time-independent studies, water-quality parameters were measured twice weekly. Instruments used were manufactured by Yellow Springs Instrument Co. (D.O. and conductivity), Orion (pH), and Scientific Products (temperature).

Chemical Analysis

Samples were taken from the test solutions at 24, 48, and 96 hr for the 96-hr study and twice weekly for the time-independent studies. Samples were placed in amber bottles with Teflon-lined caps and refrigerated until extraction and analysis. The analytical procedures follow.

For thiobencarb an aliquot of sample, ranging in volume from 100 to 750 ml, was decanted into a separatory funnel of appropriate size and extracted with two portions of dichloromethane (DCM). The volume of DCM depended on the volume of sample used. We found that a 1:8 ratio (DCM:sample) worked best. For example, if 400 ml of sample was to be extracted, then two 50-ml portions of DMB were used. A 1.0-ml portion of isooctane (2,2,4-trimethylpentane) was added to the combined extracts as a keeper. This solution was concentrated to a volume of approximately 5 ml, using a rotary evaporator at a bath temperature not to exceed 35°C. This concentrated extract was transferred to an 8-ml test tube; the rotary evaporation flask was rinsed twice with DCM to bring the volume to approximately 8 ml. This solution was further concentrated, under nitrogen gas, to a final volume of 1.0 ml. Enough internal standard, 3-amino-2,4-DNT (in DCM), was added so that "mg on column" values for 3-amino-2,4-DNT and thiobencarb were roughly equivalent.

For molinate a 200-ml portion of sample was decanted into a 250-ml separatory funnel and extracted twice with 25-ml portions of DCM. The extracts were pooled and an appropriate amount of 3-amino-2,4-DNT was added as internal standard.

All samples were analyzed using gas chromatography (Varian Model 3700) with TSD detection (nitrogen-phosphorus specific). An HP3390 integrator was used along with a Supercoport SP2250, 100/120, column. Injection volumes ranged from 1 to 5 µl at 205°C isothermal. Gas flow rates were: air, 175 ml/min; H_2, 30 ml/min; and N_2, 30 ml/min.

Statistical Analysis

Determination of LC50. To estimate the median lethal concentration (LC50), we used a computerized program developed at SRI, which is composed of several statistical methods for estimating LC50s. For this project, we used estimates derived from the binomial method when there were no partial responses and estimates from the probit method when there were partial responses.

The binomial method is valid regardless of the form of the underlying tolerance distribution and therefore gives statistically valid, but conservative, confidence intervals in all cases. It is the only appropriate method when a data set contains no partial responses. The method is a two-step process. In the first step, at each concentration level with an observed mortality of 50% or more, a significance level is computed for the hypothesis that the true mortality at that concentration is 50% or less, using only the observations at that concentration. In the second step, at each concentration level with an observed mortality of less than 50%, a significance level is computed for the hypothesis that the true mortality at that concentration is 50% or more. An estimate of the LC50 is derived as the geometric average of the adjacent concentrations with 0 and 100% mortality. The 95% confidence interval for the LC50 is the shortest interval (with limits at the concentrations or at plus or minus infinity) such that at the upper confidence limit and all higher concentrations, 50% or more of the animals have died and the significance level is 0.025 or less, and at the lower confidence limit and all lower concentrations, less than 50% of the animals have died and the significance level is 0.025 or less.

The probit method is a parametric technique that depends on the assumption that the tolerance of the organisms to the test material follows a normal distribution. The computer routine performs the probit analysis twice--once for the concentration levels expressed in linear units and once for the concentrations expressed in logarithmic units. In either case, Berkson's adjustment (one-half of a response at the highest concentration with no response and one-half of a nonresponse at the lowest

concentration with 100% response) is used when there is only one partial response.

The LC50 estimate is the maximum likelihood estimate for the mean of the tolerance distribution. The "unadjusted" confidence interval for the LC50 is derived by inverting the likelihood ratio test for determining whether any specified concentration is the LC50. A chi-square test is used to determine how well the estimated tolerance distribution fits the data (which are also plotted). In this test, adjacent concentration levels are collapsed until the expected responses (mortality and survival) are everywhere greater than 2.0. Finally, if the probability of poor fit is 0.75 or greater, a heterogeneity factor is derived from the chi-square test and the confidence interval is adjusted outward using the heterogeneity factor.

Contribution of the components of the mixture to the total toxicity. To determine whether the toxicity of the mixture of herbicides was more or less than the sum of the toxicities of the individual components, we used a modified version of a method developed by Marking and Dawson.[1] These authors calculated the sum (S) of the conributions of two compounds (A and B) to the toxicity of a mixture of the two compounds using the equation:

$$\frac{A_m}{A_i} + \frac{B_m}{B_i} = S \quad ,$$

where A_i and B_i are the individual LC50s of compounds A and B, and A_m and B_m are the LC50s of the compounds in the mixture. The values A_m and B_m are calculated by multiplying the LC50 of the mixture by the fraction contributed by each compound to the total concentration of both compounds in the mixture.

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[1]Marking, L. L., and V. K. Dawson. 1975. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals. Investigations in Fish Control Series, Report No. 67, U.S. Department of the Interior, Fish and Wildlife Service.

To explain the logic of Marking and Dawson's equation, we have rewritten it as follows:

$$S = \frac{aLC50_M}{LC50_A} + \frac{bLC50_M}{LC50_B} ,$$

where $LC50_A$, $LC50_B$, and $LC50_M$ are the experimentally derived estimates of actue toxicity for compounds A, B, and the mixture (M), respectively, and a and b are the respective fractions of compounds A and B in the mixture.

If $LC50_A$, $LC50_B$, and the amount of A and B in M are known, the theoretical concentrations of M that would kill 50% of the test organisms (under the assumption that A and B were neither antagonistic nor synergistic) can be calculated. We call that concentration the additive LC50 of M and denote it as $LC50_M$*.

On the basis of acute toxicity tests, each gram of compound B is only $LC50_A/LC50_B$ as toxic as each gram of compound A. If we assume that the toxicities of A and B are additive, each gram of the mixture M should be as toxic as a + b ($LC50_A/LC50_B$) grams of A. Thus, the additive LC50 of M is:

$$LC50_M^* = \frac{LC50_A}{a + b(LC50_A/LC50_B)} .$$

By algebraic manipulation, it can be shown that

$$S = LC50_M/LC50_M^* .$$

The statistic S ranges from zero to infinity, with a value of 1.0 denoting additivity. Because the range of S is nonsymmetric around 1.0,

Marking and dawson (1975) suggested replacing S by a corrected sum, which we will call CS, and which these authors defined as CS = 1/S-1 when $S \leq 1$ and CS = 1-S when $S > 1$.

We find little merit in this defirition and redefine CS as CS = Log S. This transformation symmetrizes the range around zero. A CS value of zero indicates additivity, a CS value of -1 corresponds to $LC\ 50_M = LC50_M^*/10$ (or synergism) and a CS value of +1 corresponds to $LC50_M = 10\ (LC50_M^*)$ (or antagonism). This transformation also simplifies the derivation of the confidence interval for CS.

Marking and Dawson (1975) derived the 95% confidence interval for CS by substituting the 95% confidence intervals for $LC50_A$, $LC50_b$, and $LC50_M$ into their equation for calculating S. We believe this procedure to be heuristic because the 95% confidence interval for CS (i.e., Log S) can be rigorously defined using the procedure described below. From the above equation,

$$\text{Log } S = \text{Log } LC50_M - \text{Log } LC50_M^* \quad ,$$

if the central limit theorem can be invoked (e.g., if Log $LC50_M$ and Log $LC50_M^*$ can be assumed to be normally distributed), then an approximate 95% confidence interval for the true corrected sum can be calculated by:

$$\text{Log } S \pm 1.96[\text{VAR}(\text{Log } LC50_M) + \text{VAR}(\text{Log } LC50_M^*)]^{1/2} \quad ,$$

where VAR denotes variance. The variance of Log $LC50_M$ can be estimated from the results of the toxicity test on the mixture. The variance for Log $LC50_M^*$ can be estimated by the equation:

$$VAR(\text{Log } LC50_M{}^*) = \left(\frac{bLC50_A}{aLC50_B + bLC50_A} \right)^2 VAR(\text{Log } LC50_B)$$

$$+ \left(\frac{aLC50_B}{aLC50_B + bLC50_A} \right)^2 VAR\ (\text{Log } LC50_A) \quad .$$

Results

Mortality of sturgeon fry exposed to thiobencarb and molinate for 96 hours is summarized in Table 2. On the basis of these data, thiobencarb appears to be considerably more toxic to white sturgeon fry than molinate. As a comparison, the 96-hr LC50 for thiobencarb was less than that for molinate by a factor of 71.

Table 2

MORTALITY OF WHITE STURGEON FRY EXPOSED TO RICE FIELD HERBICIDES FOR 96 HOURS

Chemical	Concentration (mg/L)	Number Dead (n = 10) 24 Hr	48 Hr	72 Hr	96 Hr
Thiobencarb - Control	0	0	0	0	0
	0.19	0	0	1	1
	0.27	0	2	4	4
	0.35	1	7	10	10
	0.56	3	10	10	10
	1.5	10	10	10	10
LC50 (mg/L)		0.64	0.32	0.26	
95% Conf. Limits		(0.51-0.89)	(0.28-0.36)	(0.23-0.30)	
Molinate - Control	0	0	0	0	0
	1.17	0	0	0	0
	2.13	0	0	0	0
	7.11	0	0	0	0
	12.49	1	2	2	2
	23.13	0	3	7	7
LC50		> 23.13	> 23.13	18.37	
95% Conf. Limits				(14.44-26.85)	

The mortality of sturgeon fry exposed to thiobencarb, molinate, and a mixture of the two chemicals in time-independent bioassays is shown in Table 3. After 21 to 22 days of exposure, the incipient LC50 for thiobencarb was greater than the LC50 for molinate by a factor of 12. This result is in general agreement with the results of the 96-hour bioassays, which also indicated that thiobencarb was more toxic than molinate.

Table 3

CUMULATIVE MORTALITY OF WHITE STURGEON FRY EXPOSED TO THIOBENCARB, MOLINATE, AND MIXTURE OF THE TWO IN A TIME-INDEPENDENT BIOASSAY

Treatment Level	Number Dead (n = 20)			
	Day 4	Day 7	Day 14	Final[a]
Thiobencarb - Control	0	0	0	0
3.38 µg/L	0	0	0	--[b]
6.34	0	0	1	--[b]
14.53	0	0	0	0
22.94	0	0	1	1
49.75	0	0	0	0
107.90	0	0	0	0
217.19	0	0	0	10[c]
LC50 (µg/L)	> 217.19	> 217.19	> 217.19	221.1
95% Confidence Interval				(185.5-287.0)
Molinate - Control	0	0	0	1
0.69 mg/L	0	0	3	5
1.22	0	0	1	2
2.28	0	0	4	11
3.89	0	0	11	12
6.43	2	2	18	20
LC50 (mg/L)	> 6.43	> 6.43	3.76	2.69
95% confidence Interval			(3.12-4.55)	(2.70-3.34)
Mixture - Control	0	0	0	0
0.71 mg/L	0	0	0	5
1.25	0	0	2	4
2.22	2	2	3	5
3.62	0	0	7	9
6.69	0	3	19	19
LC50 (mg/L)	> 6.69	> 6.69	4.05	3.38
95% Confidence Interval			(3.46-4.80)	(2.67-4.30)

[a] 21 Days for thiobencarb, 22 days for molinate and the mixture.

[b] These two concentrations were increased to 107.90 and 217.19 µg/L, as discussed in the text.

[c] n = 19.

The pattern of mortality in the test on the mixture was very similar to that seen in the test on molinate alone. This suggests that toxicity is not enhanced appreciably by the presence of thiobencarb. This is supported by calculating the additivity index for the joint effects of the two chemicals. That calculation results in an index of 0.13 with upper and lower 95% confidence bounds of 0.269 and -0.0009, respectively. The interactive toxicity index suggests that these two chemicals are slightly antagonistic in action; however, the effect is small, and because the confidence interval overlaps zero, we conclude that, for all practical purposes, the toxicity of the two chemicals is additive.

The water quality and analytical data for the above bioassays is appended.

Discussion

Both molinate and thiobencarb showed appreciable toxicity to white sturgeon fry. Thiobencarb was more toxic than molinate in both the 96-hr (over 70 times) and time-independent studies (over 12 times). However, molinate appeared to have a greater propensity for cumulative toxicity. As evidenced by the pattern of mortality in the time-independent study, in which the number of deaths increased over time at all concentrations. In contrast with the tests on thiobencarb, in which the LC50 obtained after 21 days of exposure was very similar to that obtained after 4 days of exposure (0.22 and 0.26 mg/L, respectively), the 22-day LC50 for molinate was approximately six times less than the 4-day LC50 (2.69 and 18.37 mg/L, respectively). Furthermore, approximately 25% mortality occurred at the lowest concentration of molinate tested, 0.69 mg, whereas no effects were discerned on fry exposed to thiobencarb concentrations of 0.11 mg/L and less.

The pattern of mortality in the joint toxicity study was very similar to the results in the concurrent study on molinate. Although the number

of deaths at a given treatment level were not as great as at the corresponding treatment level of molinate, the same indication of cumulative effects at the lower doses was apparent.

To place the results of these studies in context with environmental exposures, the actual levels of molinate and thiobencarb in the environment must be considered. According to data at the state Water Resources Control Board (Dr. John Cornacchia, personal communication), levels of thiobencarb and molinate reached 2 and 13 µg/L, respectively, in the upper Sacramento-San Joaquin Delta over a 2- to 3-week period in 1985. For thiobencarb, this value is below the incipient LC50 by a factor of about 100. It is also below by a factor of 50, a level that resulted in no mortality over a 21-day period. Thus, in the absence of additional stresses, this concentration (2 µg/L) might be expected not to produce acute effects on the fish population.

Environmental concentrations of molinate (13 µg/L) were below the incipient LC50 by a factor of about 200 and below the level that caused 25% mortality by a factor of about 50. Because we did not test concentrations below that which caused 25% mortality, it is difficult to assess the likely level of effect at 13 µg/L, but it appears that this concentration would not be expected to cause more than minimal acute effects. Our reasoning is based on the fact that the observed field concentrations (0.013 mg/L) occur only over a three-week period, which is similar to the 22-day exposure period that resulted in 25% mortality at 0.69 mg/L. Thus, it can be inferred that a concentration 50 times less than 0.69 mg/L would probably have significantly less effect over the same time period.

Considering the results of these studies and the concentrations of thiobencarb and molinate seen in the Delta, it appears that neither of these chemicals poses an acute toxicity hazard to white sturgeon fry. This is especially true when these chemicals are present in the environment for only a relatively short time. However, these results are limited to fry more than 25 days old; at earlier life stages they could exhibit greater sensitivity, which can be evaluated only by embryolarval exposures.

APPENDIX

WATER QUALITY AND ANALYTICAL DATA

Table A-1

WATER QUALITY DATA FOR THE 96-HR BIOASSAYS ON MOLINATE AND THIOBENCARB

Treatment Level	Temperature (°C)				Dissolved Oxygen (mg/L)				pH				Conductivity (µmhos)			
	$\bar{x}$	S.D.	Range	n	$\bar{x}$	S.D.	Range	n	$\bar{x}$	S.D.	Range	n	$\bar{x}$	S.D.	Range	n
Thiobencarb																
Control	15.7	0.6	15.0-16.5	5	9.4	0.3	9.1-9.8	5	7.5	0.6	7.0-8.2	5	88	25	70-105	2
1	15.9	0.4	15.5-16.5	5	9.3	0.6	8.5-9.9	5	7.6	0.7	6.7-8.5	5	83	18	70-95	2
2	16.0	0.4	15.5-16.5	5	8.8	0.7	8.0-9.9	5	7.6	0.7	6.6-8.6	5	83	18	70-95	2
3	16.0	0.4	15.5-16.5	4	9.0	0.6	8.4-9.9	4	8.0	0.4	7.6-8.6	4	105	0	--	1
4	16.1	0.3	16.0-16.5	4	9.3	0.5	8.9-9.9	3	8.0	0.5	7.6-8.6	3	130	0	--	1
5	16.1	0.3	16.0-16.5	4	9.5	0.6	9.1-9.9	2	8.0	0.7	7.5-8.5	2	115	0	--	1
Molinate																
Control	16.8	0.6	16.0-17.5	5	8.6	0.8	7.6-9.8	5	7.5	1.0	6.2-8.5	4	100	42	70-130	2
1	16.8	0.6	16.0-17.5	5	8.6	0.8	7.8-9.9	5	7.7	0.7	6.8-8.6	4	83	18	70-95	2
2	16.8	0.6	16.0-17.5	5	8.7	0.8	7.8-9.9	5	7.8	0.8	6.8-8.6	4	98	32	75-120	2
3	16.8	0.6	16.0-17.5	5	8.6	0.8	7.8-9.9	5	7.7	0.8	6.7-8.6	4	95	35	70-120	2
4	16.8	0.6	16.0-17.5	5	8.7	0.7	7.9-9.9	5	7.8	0.8	6.7-8.6	4	78	11	70-85	2
5	16.7	0.4	16.0-17.0	5	8.9	0.7	8.0-9.9	5	7.9	0.8	6.7-8.6	4	90	28	70-110	2

Table A-2

MEASURED CHEMICAL CONCENTRATIONS ASSOCIATED WITH THE 96-HR BIOASSAYS ON THIOBENCARB AND MOLINATE

Treatment Group	Measured Concentration (mg/L) $\bar{x}$	S.D.	Range	n
	Thiobencarb			
0	0	0	--	3
1	0.19	0.006	0.18-0.19	3
2	0.27	0.026	0.24-0.29	3
3	0.35	0.012	0.34-0.36	3
4	0.5[illegible]	0.028	0.54-0.58	2
5	1.[illegible]5	--	--	1
	Molinate			
0	0	0	--	3
1	1.17	0.038	1.14-1.21	3
2	2.13	0.070	2.05-2.18	3
3	7.11	0.059	7.04-7.15	3
4	12.49	0.327	12.21-12.85	3
5	23.13	0.046	23.08-23.17	3

Table A-3

WATER QUALITY DATA FOR THE TIME-INDEPENDENT BIOASSAYS ON THIOBENCARB, MOLINATE, AND A MIXTURE OF THE TWO

Chemical	Treatment Level	Temperature (°C)					Dissolved Oxygen (mg/L)					pH					Conductivity (µmhos)				
		$\bar{x}$	SD	n	Range		$\bar{x}$	SD	n	Range		$\bar{x}$	SD	n	Range		$\bar{x}$	SD	n	Range	
Thiobencarb	0	18.0	0.0	12	18.0	18.0	7.2	1.6	12	6.0	11.2	6.6	0.1	12	6.4	6.7	63.8	12.5	4	45.0	70.0
	1	18.0	0.0	12	18.0	18.0	7.4	1.8	12	5.9	11.4	6.5	0.2	12	6.1	6.6	60.0	10.8	4	45.0	70.0
	2	18.1	0.3	12	18.0	19.0	7.3	1.7	12	6.4	11.4	6.5	0.2	12	6.1	6.7	58.8	10.3	4	45.0	70.0
	3	18.8	0.4	12	18.0	19.0	7.4	1.8	12	6.3	11.6	6.5	0.2	12	6.0	6.7	56.3	12.5	4	40.0	70.0
	4	18.8	0.4	12	18.0	19.0	7.1	1.3	12	5.8	11.4	6.6	0.2	12	6.1	6.8	63.8	14.9	4	45.0	80.0
	5	18.8	0.4	12	18.0	19.0	7.4	1.6	12	6.3	11.6	6.6	0.3	12	6.1	7.0	62.5	6.5	4	55.0	70.0
Molinate	0	18.5	0.5	12	18.0	19.0	7.1	1.2	12	6.3	10.2	6.5	0.2	12	6.1	6.7	63.8	12.5	4	45.0	70.0
	1	18.5	0.5	12	18.0	19.0	7.2	1.5	12	6.1	10.8	6.5	0.2	12	6.1	6.7	63.8	12.5	4	45.0	70.0
	2	18.5	0.5	12	18.0	19.0	7.0	1.3	12	6.2	10.4	6.6	0.2	12	6.1	6.7	66.3	12.5	4	50.0	80.0
	3	18.5	0.5	12	18.0	19.0	6.9	0.9	12	6.2	9.0	6.6	0.2	12	6.1	6.8	60.0	10.8	4	45.0	70.0
	4	18.5	0.5	12	18.0	19.0	6.8	1.4	12	4.4	10.6	6.6	0.2	12	6.2	7.0	56.3	11.1	4	45.0	70.0
	5	18.5	0.5	12	18.0	19.0	7.3	2.1	12	5.1	11.8	6.7	0.2	12	6.2	6.9	58.8	13.2	4	50.0	70.0
Mixture	0	18.6	0.5	12	18.0	19.0	7.0	1.4	12	6.0	11.2	6.6	0.1	12	6.4	6.7	56.3	7.5	4	45.0	60.0
	1	18.5	0.5	12	18.0	19.0	7.2	1.7	12	5.9	11.2	6.6	0.1	12	6.4	6.7	66.3	12.5	4	60.0	85.0
	2	18.5	0.5	12	18.0	19.0	6.7	1.3	12	5.2	10.6	6.6	0.1	12	6.4	6.7	55.0	7.1	4	45.0	60.0
	3	18.6	0.5	12	18.0	19.0	6.4	0.8	12	5.0	8.2	6.6	0.1	12	6.4	6.7	56.3	7.5	4	50.0	65.0
	4	18.6	0.5	12	18.0	19.0	5.8	1.9	12	4.6	9.0	6.6	0.1	12	6.4	6.7	57.5	5.0	4	50.0	60.0
	5	18.6	0.5	12	18.0	19.0	7.1	2.1	12	5.8	11.8	6.6	0.1	12	6.5	6.8	53.8	7.5	4	45.0	60.0

Table A-4

ACTUAL CONCENTRATIONS ASSOCIATED WITH TIME-INDEPENDENT STUDIES ON THIOBENCARB, MOLINATE, AND A MIXTURE OF THE TWO CHEMICALS

Chemical	Treatment Level	Measured Concentrations			
		x	SD	n	Range
Thiobencarb (µg/L) (alone)	0	0.00	0.00	9	--
	1	3.38	0.37	5	2.87- 3.64
	2	6.34	0.90	5	5.20- 7.72
	3	14.53	1.41	9	12.28- 15.42
	4	22.94	2.21	9	19.47- 26.21
	5	49.75	5.38	9	39.18- 55.83
	6	107.90	11.43	4	93.81-121.24
	7	217.19	25.98	4	191.49-243.05
Molinate (mg/L) (alone)	0	0.00	0.00	6	--
	1	0.69	0.04	6	0.62-0.72
	2	1.22	0.03	6	1.19-1.28
	3	2.28	0.10	6	2.17-2.39
	4	3.89	0.13	6	3.78-4.07
	5	6.43	0.25	6	6.10-6.73
Thiobencarb (µg/L) (in mixture)	0	0.00	0.00	6	--
	1	3.52	0.27	6	3.19- 3.88
	2	7.10	0.74	6	6.25- 8.16
	3	13.15	1.87	6	11.62-16.43
	4	24.99	3.25	6	21.04-28.66
	5	46.38	4.50	6	41.38-54.70
Molinate (mg/L) (in mixture)	0	0.00	0.00	6	--
	1	0.71	0.01	6	0.69-0.73
	2	1.24	0.03	6	1.20-1.27
	3	2.21	0.06	6	2.11-2.28
	4	3.60	0.04	6	3.57-3.62
	5	6.64	0.27	6	6.53-7.20

APPLNDIX G

Survival, Growth, Metal Accumulation, and Bone Development of Young Striped Bass Exposed to Copper or Cadmium

Final Report

Prepared for:

State of California
State Water Resources Control Board
Toxic Substances Control Program
Contract Manager: Dr. John Cornacchia

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Prepared by:

U.S. Fish and Wildlife Service
Columbia National Fishery Research Laboratory
Project Director: Wilbur Mauck

Principal Investigator: Tom Jackson, Leader
Denver Field Research Station

Analytical Support: Ray Wiedmeyer, CNFRL
Chemistry Section

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Summary

Acute and chronic toxicity tests were conducted with striped bass larvae and juveniles from Sacramento-San Joaquin stocks, to determine concentrations of copper and cadmium which adversely affect early survival, growth, and development. All tests were performed with background water quality conditions approximating those in striped bass nursery habitats of the San Francisco Bay-Delta Estuary.

The acute toxicity of copper and cadmium varied with the age and stock of fish tested. LC50 estimates for 96-hour tests with copper ranged from 36 µg/l for 3-day-old larvae to 463 µg/l for 9-day-old fish. Cadmium LC50 estimates ranged from 6 µg/l for larvae to 1,010 µg/l for juveniles. Acute toxicity of both metals to larvae decreased during the first 11 days after hatching and then increased through day 20 post-hatch. The behavioral response of larvae to lethal metal exposure was a progressive decline in activity leading to terminal immobilization.

Striped bass larval survival was significantly reduced at 50 µg/l after 15 and 30 days exposure to copper in chronic toxicity testing. Mortality during these periods was proportional to copper concentration. However, a large fraction of the exposed fish were larvae which failed to inflate the swim bladder. Mortality of uninflated fish after day 30 in treated and control test chambers masked the effects of copper exposure on larval survival. Copper concentrations of 3-50 µg/l had no significant effect on the growth of treated larvae. Juvenile striped bass survival was significantly reduced at 100 µg Cu^{++}/l after 15 days in a second chronic test with copper. Mortality thereafter was negligible, indicating probable acclimation to copper by treated fish. Levels of copper below 100 µg/l had no effect on juvenile survival. Growth of test fish was unaffected by copper exposure over the range tested (6.3 - 100 µg/l).

Cadmium concentrations of 0.6 - 10 µg/l had no effect on survival or growth of juvenile striped bass exposed for 60 days.

Copper residues in striped bass from chronic toxicity tests were proportional to copper dosage only for the upper range of concentrations tested (25 - 100 µg/l). In both chronic tests with copper, only fish from the highest test concentrations had copper residues significantly greater than control fish residues. Bioconcentration factors (BCF) for copper ranged from 16 to 227 and were highest for control fish. Cadmium residues in striped bass after a 60-day exposure were directly proportional to cadmium concentrations in water. Residues averaged 0.007 µg/g (wet weight) in control fish and 0.679 µg/g for juveniles exposed to 10 µg/l. The cadmium content of all treated fish was significantly higher than control levels. Cadmium BCF's were consistently low, with the entire range (50 - 80) represented in the replicate control fish groups.

Biochemical and mechanical characteristics of vertebral bone from copper-exposed fish in chronic tests, were not consistently related to copper concentrations in water. Calcium content was significantly reduced in early juveniles treated at 6.3 - 50 µg Cu^{++}/l but was unaffected in older juveniles tested at 6.3 - 100 µg Cu^{++}/l. Chronic exposure to cadmium had no effect on bone characteristics analyzed from treated fish.

Striped bass are susceptible to toxic effects from copper or cadmium exposure during the larval and early juvenile stages when relative year class size is established. More information is needed to determine the actual concentration and bioavailability of these metals in estuarine nursery habitats to accurately assess effects on striped bass recruitment.

Introduction

Declining numbers of striped bass Morone saxatilis in the Sacramento-San Joaquin Delta over the past 20 years, have prompted efforts to determine the cause(s) for observed decreases in this important fishery (Eldridge et al. 1981; SWBG 1982; Stevens et al. 1985). Environmental contaminants in the Sacramento-San Joaquin system have been suspected as contributing to this decline and have received some attention (Jung and Bowes 1980; Jung 1981; Whipple et al 1983). However, there is little information concerning pollutant impacts on the early life history stages of striped bass, particularly for the first 60 days post-hatch, during which relative year-class strength is thought to be established Chadwick et al. 1977; Stevens 1977). Such information is crucial to accurate assessments of contaminant-related changes in Bay-Delta striped bass populations.

Copper and cadmium are major inorganic contaminants of the Sacramento-San Joaquin Delta, which originate from several sources including municipal sewage effluents (Jung and Bowes 1980), urban runoff (Cole et al. 1984) and agricultural and mine drainage. These heavy metals have been found in upstream and Delta water samples at concentrations exceeding levels considered detrimental to aquatic life (California State Water Resources Control Board, unpublished data). Both metals are present at concentrations reported acutely toxic to larval and juvenile striped bass, although most previous toxicity tests have been conducted in freshwater (Summarized by Rogers et al. 1980). The toxicity of copper and cadmium to young striped bass in more recent studies (Mehrle and Ludke 1983; Wright et al. 1985) has been shown to vary with background water quality. As a result, toxicity tests on young striped bass under water quality conditions approximating those of the Bay-Delta, need to be performed with these metals.

The need for information on declining striped bass stocks led to the present Cooperative Agreement between the California State Water Resources Control Board and the U.S. Fish and Wildlife Service. The Agreement is intended to provide data on the toxic effects of cadmium and copper on young striped bass. This report is a final compilation of toxicity test results from experiments conducted in 1984 and 1985.

Methods

Flow-Through Toxicity Tests

During 1984, flow-through acute toxicity tests with striped bass were conducted in accordance with ASTM (1980) guidelines, using proportional diluters (Mount and Brungs 1967) with modifications suggested by Benoit and Puglisi (1973). Partial life-cycle (60-day) toxicity tests were also performed with these diluters following methods developed by the Fish and Wildlife Service (Mehrle and Ludke 1983) for toxicological experiments with young striped bass. Design of chronic tests also included recommendations of Feder and Collins (1982).

Acute and chronic tests included five duplicate concentrations of the toxicant with a dilution factor of 0.5 between concentrations, plus duplicate controls. Each experimental chamber (25 cm x 58 cm glass tank x 27.6 cm glass standpipe with fine-mesh screen = 40 l volume) received 1.0 liter of test solution at each diluter cycle, with 6 cycles per hour. The test solution delivered to all tanks included 5%. salinity as Instant Ocean^{R} added to charcoal-dechlorinated Denver tapwater. Toxicant-treated tanks also received a concentration of Cu^{++} as $CuSO_4 \cdot 5H_2O$ (Analytical Reagent) or Cd^{++} as $CdCl_2 \cdot 2\frac{1}{2}H_2O$ (analytical Regent) as an aqueous solution metered into the diluter using an automated pipette (Micromedic^{R} Model 25000, with glass dispensing pumps).

Static Toxicity Tests

In 1985, static acute toxicity tests with larval striped bass were conducted to increase the range of concentrations and

fish ages/sizes tested with copper and cadmium. Each of these tests included seven copper or cadmium concentrations plus a control with a test solution volume of 1.5 liters in 2.0 liter glass (Pyrex^{R}) containers. Diluent water quality for these tests was 1.0, 2.0, or 5.0%. salinity as Instant Ocean^{R} added to dechlorinated, degassed (<95% saturation as total gas) tapwater. One freshwater test with copper and one with cadmium were also performed using tapwater (<100 mg/liter TDS) without added salt as the diluent. Copper and cadmium test concentrations were prepared by adding the required volume of an aqueous stock solution of either metal to the various test containers using volumetric pipettes. Water temperature for these tests was maintained at 18 ± 1 C by immersing the test containers to the depth of the test solution, in temperature-regulated water baths.

Water Quality Monitoring

Temperature for all acute and chronic tests was maintained at 18 ± 1 C by adjusting the mix of hot (48 C) and cold (12 C) water coming into the diluters (1984 tests) or water baths (1985 tests) through mixing valves. Photoperiod for all tests was ambient daylight over the interval of each test and increased for the entire period of testing: April 27 to September 13, 1984, and May 7 to June 10, 1985.

Physical and chemical conditions monitored during acute toxicity tests included temperature (mercury thermometer), pH (Beckman-Altex Model 3560 pH meter), dissolved oxygen (YSI Model 54 A dissolved oxygen meter), total dissolved solids (gravimetric analysis of total residue on evaporation at 103 C), and total hardness [EDTA - APHA (1980)]. Temperature was checked daily during acute tests while the remaining parameters were determined once during each experiment from a random selection of three test containers. Water quality monitoring was conducted just before and after acute tests with early larvae, to minimize disturbance of the fish as a potential cause of mortality during the test.

For chronic toxicity tests, all of the above physicochemical factors were monitored along with ammonia (Orion^{R} Model 95-12

ammonia electrode). Temperature was checked daily and the remaining factors were determined weekly from three test containers selected at random. In addition, biweekly water samples were collected in acid rinsed polyethylene bottles from all test containers during chronic studies. These samples were acidified to pH$\leq$ 2.0 with Ultrex^{R} nitric acid and subsequently analyzed for total copper or total cadmium by graphite furnace atomic absorption using U.S.E.P.A. Standard Furnace Methods 220.2 for copper and 213.2 for cadmium.

During the entire striped bass testing period, the effectiveness of the laboratory dechlorination system (two pressurized beds of activated carbon) was periodically checked by testing for total residual chlorine (TRC) in the finished water. Colorimetric analyses (La Motte 4076, orthotolidine colorimeter) of finished water samples consistently showed TRC levels <0.05 mg/l (limit of detection = 0.02 mg/l).

Test Fish Maintenance

Test organisms for all toxicity tests were striped bass obtained as 24-48 hour post-hatch larvae (1984) or 20-48 hour post-fertilization eggs (1985) from the California Department of Fish and Game Central Valley Hatchery, Elk Grove, California. These fish were progeny of adult striped bass collected by electrofishing from the Sacramento River. Adult fish were artificially spawned after induced maturation by hormone (HCG) injection. The eggs and larvae were transported by air between Sacramento, CA, and Denver, CO, in groups of 15,000-20,000 in approximately 10 l of water contained in sealed plastic bags partially inflated with pure oxygen. Each bag was enclosed in a styrofoam box with an outer cardboard shell for insulation and protection. Maximum elapsed time between larval packaging at the Central Valley Hatchery and larval release into holding tanks at the Denver Federal Center, was 8 hours.

Striped bass eggs received in 1985 were held in cylindrical incubation tanks with 53 liter volumes and conical bottoms, similar to tanks used by Carlberg et al. (1984). These

incubators were partially immersed in rectangular water baths maintained at 18 C. A maximum of 20,000 eggs per tank (377 eggs per liter) was incubated in freshwater with an upwelling water current maintained by mild aeration. After hatching, water in the incubators was renewed each day with 240 liters of clean water gravity-fed from head tanks at 1-2 liters per minute [99% replacement rate based on the exchange formulas of Kraul et al. (1985)]. Fine-mesh nylon screens fitted over the base of polyethylene funnels, were positioned in the outflow port at the perimeter of each incubator to retain eggs, larvae, and brine shrimp nauplii in the incubator under flow-through conditions. At 12-24 hours after hatching, the salinity of incubation water was increased to 1%. with Instant Ocean® and 24-72 hours thereafter, raised to 2%. or 5%. as needed for acute toxicity tests at different salinities. One group of larvae was held entirely in freshwater for comparison. At 25-30 days post-hatch, all larvae were transferred to rectangular rearing tanks and maintained as described below.

All striped bass larvae for toxicity testing in 1984 and 25-30 day-old larvae in 1985 were maintained in rectangular fiberglass tanks with a water volume of 227 liters. Initial stocking density in the holding tanks was 88 fish per liter in 1984 and 84 fish per liter in 1985. Constant water volume in each tank was regulated by a standpipe at one end fitted with a fine-mesh nylon screen to prevent the escape of larvae or live food organisms. Mild aeration was provided in each tank through two cylindrical (1.5 cm diameter x 2.5 cm long) air stones receiving filtered, compressed air regulated by toggle-type gang valves. Aeration rate was adjusted to produce a water circulation velocity of 1-2 cm/sec associated with the upwelling bubble stream. Holding tanks were siphoned daily to remove debris.

In 1984, holding tanks received 120 liters of clean water each day, which replaced 42% of the tank volume from the exchange formulas of Kraul et al. (1985). Replacement water was aerated

for 18-24 hours before being pumped or gravity-fed into holding tanks, to equilibrate dissolved gas content with atmospheric conditions. Salinity in holding tanks and replacement water was increased from 1%. to 5%. Instant Ocean® at 2%. per day commencing 48 hours after the larvae were received. Replacement water volume in 1984 was increased from 120 to 360 l/day (80% daily replacement) beginning 30 days after the fish were received as larvae. In 1985, daily replacement volume was 480 liters or 99% exchange (Kraul et al. 1985). Water temperature of the holding tanks was maintained at 19±2 °C by ambient room temperature. Illumination in the holding tanks was indirect because the tanks were removed from natural light and were shaded from overhead fluorescent lighting in the laboratory. The artificial photoperiod was 12 hours per day.

Temperature and dissolved oxygen were monitored daily in the holding tanks and total gas saturation (Weiss saturometer) was determined each day in replacement water tanks, at the completion of each 18-24 hour aeration period (prior to use of the water in holding tanks) and at the beginning of the next aeration period. Total gas saturation was also periodically determined in water siphoned from the holding tanks. Ammonia and pH in the holding tanks were determined infrequently because of consistent (pH) and consistently low (ammonia) readings during initial monitoring.

In addition to physical and chemical monitoring, holding tanks were also sampled daily to monitor swimbladder inflation and other morphological changes among striped bass larvae. From 5 to 25 days post-hatch, daily samples of larvae were examined microscopically to check the rate of swimbladder inflation and determine the percentage of normally developed fish.

Striped bass larvae, fry and fingerlings in the holding tanks were fed daily with brine shrimp (Artemia salina) nauplii at a target density of 5 nauplii per milliliter of tank water. Nauplii were harvested by filtration from 48 hour cultures of decapsulated brine shrimp cysts (San Francisco Bay®) incubated in a saturated brine (NaCl) at 27 C. Feeding commenced at 5 days

post-hatch and continued until holding tank fish had been completely distributed for toxicity tests. During flow-through acute and chronic toxicity tests, striped bass were also fed brine shrimp nauplii metered into each diluter mixing box with a multi-channel peristaltic pump (Masterflex^R Model No. 7568), from a stock suspension of nauplii in 10%. Instant Ocean^R. Target density in each test container was 1 nauplius per milliliter of test solution. In the holding tanks and during chronic toxicity tests, striped bass were also fed Silver Cup^R salmon starter three times daily, commencing at 50 days post-hatch for all fish.

Transfer of larvae and fry to toxicity test containers was conducted without removing fish from the water. Groups of striped bass were dipped from a holding tank in a glass beaker, and then released into a test container after being counted. This was done to minimize physical trauma and stress. Fish at 40 days post-hatch and older were netted from holding tanks with fine-mesh aquarium dip nets because their swimming ability precluded easy capture in beakers. Loading factors for static and flow-through tests were calculated as grams of fish per liter of test container volume. Flow-through loading factors based on flow rates (grams of fish per liter of flow per day) were also calculated.

Experimental Design

Flow-through acute toxicity tests conducted in 1984 were designed to provide LC50 estimates and 95% confidence limits based on fish mortality in five duplicate toxicant (metal) concentrations, plus duplicate controls. The concentrations were arranged as a linear series separated by a dilution factor of 0.5. LC50 estimates and 95% confidence limits were calculated by the probit method (Finney 1971; Stephan 1977). Static acute tests in 1985 were also analyzed for LC50 estimates by the probit method but the range and interval of test concentrations were adjusted to maximize the number of partial kills over 96 hours rather than conform to mathematical or other conventions (Stephan 1977).

Experimental design of 60-day chronic toxicity tests was a completely randomized design for mortality data, in which the test container was the experimental unit observed for a response, i.e. percent mortality (See Type I Experimental Design of McClave et al. 1981). Mortality was recorded daily in all containers (aquaria), and analyzed in relation to copper or cadmium concentration by conducting an analysis of variance (AOV) on the arcsin transformation for proportions (Snedecor 1965; angle = % mortality). For tests with significant treatment effects according to AOV, a multiple means comparison test (Duncan's) was used to compare average percent mortality between toxicant concentrations. In addition, a combined AOV - linear regression analysis (Sokal and Rohlf 1969, Section 14.6) was performed to determine whether cumulative mortality was linearly related to exposure time at each test concentration. Slopes of the resulting regressions (one for each test concentration) were then compared (Sokal and Rohlf 1969, Section 14.9) to test for significant differences in cumulative mortality rate between concentrations.

The experimental design of growth data collection from chronic tests followed Type 2 Experimental Design of McClave et al. (1981) for which each individual fish is an experimental unit with an observed quantitative response (growth). For each chronic test, data on growth as length (mm) and weight (mg or g) were determined from a sample of fish collected for biochemical (RNA/DNA and bone development) analyses at 15 and 30 days after treatment began. These data were analyzed by AOV as a balanced, nested design because the number of fish sampled from each container was constant. Growth data collected at the completion of each test, were analyzed by AOV as a completely randomized, unbalanced design due to differences among concentrations in the number of surviving fish. This was done after testing the necessary assumption that within-tank variability in fish growth approximately equaled between-tank variation, by comparing these variances using a nested AOV model (McClave et al. 1981).

An additional analysis of growth was performed by comparing the slopes of weight on length regressions for the fish at each concentration, at each time interval (15 days, 30 days, and end of test = 48 or 60 days), using the method of Sokal and Rohlf (1969, Section 14.9).

Maximum Acceptable Toxicant Concentration (MATC) values were determined for each chronic toxicity test as the highest exposure concentration which had no observable adverse effect on striped bass survival or growth by comparison with control fish [See Review by Buikema et al. (1982)]. However, ratios between acute and chronic toxicity [ACR's (Kenaga 1982)] were not calculated because of the large variation in metal acute and chronic toxicity associated with changes in striped bass age/size.

Quality assurance in data collection and analysis followed guidelines developed within the Columbia National Fishery Research Laboratory. All data were recorded on paper and independently checked for accuracy and completeness by two or more research personnel. Data input for statistical analysis by computer was also double checked for accuracy with original data sheets. Data sheets, computer input records and computer output hardcopy are all maintained in secure files for re-examination and analysis as needed.

Copper and Cadmium Analysis Water

Water samples collected during Chronic Toxicity Test #'s 1, 2, and 3 were analyzed for total copper or total cadmium after acidification and storage at room temperature. Prior to analysis, each sample was examined for the presence of silicates or other insoluble materials and for background or matrix interference. None of the water samples contained insoluble matter or interference, so filtration, digestion, and addition of modifiers were not required for sample preparation.

Calibration standards were prepared at the time of analysis by diluting copper or cadmium stock solutions to contain 1% (V/V) HNO_3 and to bracket concentrations present in water samples. Prepared samples were then analyzed with a Perkin-Elmer 5000 AA

unit equipped with a graphite furnace and an AS-1 autosampler. The following list of instrument parameters indicates the instrument operating conditions during analysis of all samples:

		Cu^{++}	Cd^{++}
1.	Drying Time & Temp. :	35 sec - 200 C	40 sec - 200 C
2.	Ashing Time & Temp. :	30 sec - 1000 C	30 sec - 400 C
3.	Atomizing Time & T. :	7 sec - 2600 C	5 sec - 1800 C
4.	Purge Gas :	Argon	Argon
5.	Wavelength :	327.4 nm	228.8 nm
6.	Injection Volume :	10 µl	10 µl
7.	Response Units :	Peak Area	Peak Area

Copper and cadmium concentrations in water samples were determined using external standards and a calibration curve. Peak area data from the AA were transferred to a P-E data station and reduced using a linear regression program. A 4 point calibration curve with a required minimum $R^2 = 0.999$, was used for all quantitation.

Quality control for sample analysis consisted of one reagent blank, two spiked samples, an NBS reference material, and two standards per 12 water samples. The AA signal was always optimized so that and sensitivity for Cu^{++} and Cd^{++} exceeded the manufacturer's recommendations.

Tissue Residue Analysis

At the conclusion of each chronic toxicity test, a sample of surviving fish was removed from the duplicate test containers for each toxicant concentration and the control. In most cases, this sample represented more than 50% of the survivors in each test tank. Total length (mm) and weight (mg or g) were determined for individual fish in all samples and the samples were individually frozen (-23 C) in air-tight plastic bags labeled with pertinent sample information.

Within 60 days after collection, frozen samples were transported by air from Denver, Colorado, to Columbia, Missouri, for copper or cadmium residue analysis by the Columbia National Fishery Research Laboratory's Chemistry Section. The samples

were packed in sufficient dry ice to keep them solidly frozen in transit. Upon arrival, all samples were logged by code and stored at -28 C prior to final preparation for analysis.

Sample preparation began with a wet weight determination followed by freeze drying to constant weight in 0.5 liter Whirl-pak sample bags. Constant weight was achieved for all samples within 6 days of drying time. After dry weight and percent moisture determinations, freeze-dried samples were homogenized and then stored in sealed Whirl-pak bags in a desiccator until time of analysis.

For analysis, 0.5 g of each freeze-dried sample was digested with 3 ml concentrated HNO^3 added to the sample in a 40 ml pressure digestion tube, and vortexed (20 sec) to produce a thick slurry. The sample was then covered with plastic film (Saran Wrap), allowed to digest for 3 hours in a hood, and then digested samples were rinsed with U-P water into tared 100 ml polyethylene bottles and made up to 100 ml with additional U-P water.

Calibration standards were prepared at the time of analysis by diluting stock solutions to contain 3% HNO_3 (v/v) and to bracket sample concentrations of copper or cadmium.

Instrument parameters for the Perkin-Elmer 5000 AA system used for tissue residue analyses, were identical to those indicated for water analysis above. Analytical methods for determining metal residues in fish were identical to those of Lowe et al. (1985).

Copper and cadmium concentrations in digested tissue samples were quantified using external standards and a three point calibration curve determined by least squares fit to the standards data. Addition of modifiers or standards during the tissue analyses was not necessary because the tissue matrix presented no problems of signal suppression/enhancement, background interference, or memory effects.

Tissue residues of copper and cadmium were compared with water concentrations and with the mean weight of fish from each toxicity test, by linear regression analysis coupled with one-way analysis of variance (Sokal and Rohlf 1969, Section 14.6). This

was done to evaluate any trends between residue levels and factors likely involved in residue accumulation. Steady state bioconcentration factors or BCF (Hamelink 1977; Veith et al. 1979; Zitko 1980) were determined for each residue sample representing each replicate metal concentration and control from the chronic toxicity tests. The equation $BCF = C_F/C_W$ was used (C_F = residue in fish; C_W = concentration in water) because tissue and water concentrations appeared linearly related, a necessary condition for BCF determination by this equation (Veith et al. 1979).

Mechanical and biochemical Analyses

At the completion of each chronic toxicity test, a sample of four fish was removed for bone development analyses. Parameters monitored to depict overall bone integrity included mechanical properties, bone density, and biochemical composition. Fish from each sample were weighed, measured, and frozen (-23 C) in individual glass vials prior to analysis. Within 90 days after sampling, these fish were X-rayed prior to dissection and removal of vertebrae 3-10 (counted from the skull) for compressive loading tests to determine bone strength, elasticity and toughness, as measures of structural/mechanical integrity (Hamilton et al. 1981). Any deformed vertebrae indicated on radiographs were excluded from bone development analyses. No skeletal deformities were observed among the fish sampled.

Following mechanical testing, biochemical composition and vertebral density were determined. Biochemical factors analyzed included collagen (Flanagan and Nichols 1962), hydroxyproline (Woessner 1961), and proline (Troll and Lindsley 1955) as major organic matrix constituents of bone, plus calcium (Gitelman 1967) and phosphorus (Fiske and Subbarow 1925) as principal mineral components. Vertebral density was calculated as the mean dry weight (mg) of eight vertebrae from each fish divided by mean vertebral volume (cm^3).

RNA and DNA content of whole fish were determined from samples of three fish collected at 15 days, 30 days, and at the completion of each chronic toxicity test. Nucleic acids were

extracted from cold aqueous homogenates and analyzed for RNA and DNA concentration by estimation, from absorbency of the proper hydrolyzate at 260 nm (Buckley 1979; Munro and Fleck 1966). RNA/DNA ratios were then compared between toxicity test concentrations for the sampling periods.

Data on vertebral mechanics and biochemistry and RNA/DNA ratios were subjected to one-way AOV. As warranted, treatment means from the different toxicity test concentrations were then compared by Duncan's ne multiple range test.

Mechanical and biochemical analyses of bone development were performed by the same procedures, personnel and equipment used to monitor bone development in other striped bass stocks (Mehrle et al. 1982). RNA/DNA data collection followed the methods of Buckley et al. (1985).

Results

General

Five shipments each containing an estimated 20,000 striped bass larvae (24-48 hour post-hatch) were transported to Denver by air cargo from various U.S. sources, between March 19 and June 1, 1984. Larvae obtained from St. Johns River, Florida (March 19), and Coos River, Oregon (June 1) stocks, died within 3 days after introduction into holding tanks. The probable causes of this mortality are:

St. Johns River - larvae were 48 hours post-hatch at the time of shipment and therefore entering a generally accepted period of extreme sensitivity to handling stress; shipping container was externally damaged and had been transported upside down as evidenced by a large number of larvae trapped in the folds of the top of the plastic bag containing the fish.

Coos River - ova used to produce these larvae were reported as over-mature at the time of fertilization, due to erratic fluctuations in river water temperatures to which

spawning adults were subjected; complete mortality of transported fish in Denver coincided with complete mortality of larvae from this same lot which were incubated at a site near Coos River.

Larvae (24-hour post-hatch) obtained on April 14, 1984, from Santee-Cooper River stocks in South Carolina, survived well in a holding tank immediately following transport, but sustained approximately 25% mortality after 10 days in the holding tank. On April 27, most active, feeding larvae were removed for an acute toxicity test with copper. Swimbladder inflation rate among remaining fish was less than 5% so all these larvae were discarded on May 5, 1984.

Striped bass larvae (24-30 hours post-hatch) from Sacramento-San Joaquin stocks were received on May 2 and May 17, 1984. The first group of larvae survived well in a holding tank (less than 10% mortality over 30 days), and had an estimated 25-30% swimbladder inflation rate at 15 days post-hatch. This group of striped bass was the source of fish for all of the 1984 tests reported here except the flow-through acute toxicity test with copper. The larvae received on May 17 sustained approximately 60% mortality over the first 10 days in a holding tank. By day 15 post-hatch, less than 5% of the surviving larvae had inflated swimbladders, and total mortality had increased to about 85% so the remainder of the lot was discarded.

For tests in 1985, Sacramento-San Joaquin striped bass eggs (20-48 hours post-fertilization) were received in lots of 20,000 each on April 27, and May 10 and 24, from the Central Valley Hatchery, Elk Grove, California. Hatching rate for these embryos was nearly 100 percent, and hatching was completed 24-48 hours after arrival in Denver. Survival of larvae in all three groups exceeded 95% during the first 30 days post-hatch. Swimbladder inflation rates 15 days after hatching ranged from 22% to 30% among the three groups of fish.

Holding Tank Water Quality

Water quality parameters monitored in striped bass holding tanks were relatively uniform over the entire experimental period

(Table 1). Average dissolved oxygen concentrations were consistently greater than 80% from April to July, although individual readings ranged as low as 47% (3.4 mg/l) in May, July, and August, due to short-term laboratory power outages which temporarily interrupted holding tank aeration. Other physical and chemical parameters determined in the holding tanks, were within the range of conditions generally considered desirable for the growth and survival of young striped bass (Table 1).

Table 1. Water quality of striped bass holding tanks.

Month	Sample Size	Temp. (C) Mean/Range	D.O.(mg/l) Mean/Range	%Saturation[a] D.O./Tot.Gas		pH Mean	TDS %.	NH_3N mg/l
April	20	19.5/17-21	6.6/4.7-8.0	88	100.3	7.4	4.8-5.3	0.01
May	26	21.4/19-24	5.8/3.4-8.6	80	99.0	7.4	4.6-5.1	0.01
June	25	21.9/19-23	5.9/4.5-7.3	82	-	7.5	4.8-5.5	0.01
July	28	21.8/20-24	5.8/3.4-7.4	80	-	-	-	-
August	26	21.1/19-23	5.4/3.5-7.4	74	-	-	-	-

[a] Computed according to Colt (1983).

Acute Toxicity

Conditions for acute toxicity tests of striped bass exposed to copper or cadmium are summarized in Table 2. Loading factors and other conditions were well within the guidelines for flow-through and static toxicity testing (ASTM 1980).

As expected, the acute toxicity of cadmium was considerably greater (about 7-fold) than that of copper for early post-hatch striped bass (Compare Tables 3 and 4). Mortality of striped bass was directly proportional to metal concentration as indicated by

Table 2. Acute toxicity test conditions for flow-through (1984) and static (1985) tests with striped bass.

Test	Date of Test	Toxicant	Fish Age Days	Fish Wt. mg.	Fish per Tank	Loading Factors g/l - g/l/d		Temp. (C)	D.O. mg/l	pH	Hardness mg/l	TDS %.
1984 (Flow-Through)												
A	4/27/84	$CuSO_4$	14	2.3	200	0.01	0.003	19	6.2	7.4	226	4.8
B	5/21/84	$CdCl_2$	20	4.1	20	0.002	0.0006	19	6.7	7.6	214	4.2
C	9/6/84	$CdCl_2$	128	6800	10	1.7	0.47	19	5.8	7.4	239	5.0
1985 (Static)												
1	5/28/85	$CuSO_4$	3	0.7	20	0.009	-	18	7.1	7.0	16	<0.1
2	5/14/85	$CuSO_4$	3	0.6	20	0.008	-	18	7.4	7.1	51	1.0
3	5/7/85	$CuSO_4$	9	1.1	20	0.015	-	18	7.2	7.4	241	5.0
4	5/21/85	$CuSO_4$	10	1.0	20	0.013	-	18	7.1	7.2	96	2.0
5	6/5/85	$CuSO_4$	11	0.9	20	0.012	-	18	7.4	7.1	48	1.0
6	5/28/85	$CuSO_4$	17	1.2	20	0.016	-	18	7.2	7.3	101	2.0
7	5/28/85	$CdCl_2$	3	0.7	20	0.009	-	18	7.0	7.0	17	<0.1
8	5/14/85	$CdCl_2$	3	0.6	20	0.008	-	18	7.4	7.1	53	1.0
9	5/7/85	$CdCl_2$	9	1.1	20	0.015	-	18	7.1	7.4	237	5.0
10	5/21/85	$CdCl_2$	10	1.0	20	0.013	-	18	7.2	7.2	98	2.0
11	6/5/85	$CdCl_2$	11	0.9	20	0.012	-	18	7.3	7.1	48	1.0
12	5/28/85	$CdCl_2$	17	1.2	20	0.016	-	18	7.4	7.3	102	2.0

Table 3. Acute toxicity of copper to 14-day-old striped bass (Test A).

	Response - Cumulative % Mortality (Actual Mortality)			
Dose Copper (µg/l)	Time (hours) 24	48	96	168
250 A	22.5 (45)	73.5 (147)	100 (200)	-
250 B	25.5 (51)	76.5 (153)	100 (200)	-
125 A	19.5 (39)	61.5 (123)	93 (186)	100 (200)
125 B	34.5 (69)	63.5 (127)	93 (186)	100 (200)
62.5 A	16.0 (38)	55.0 (110)	77.5 (155)	93.5 (187)
62.5 B	17.0 (34)	64.0 (128)	76.5 (153)	92.0 (184)
31.3 A	3.0 (6)	11.5 (23)	19.5 (39)	27.5 (55)
31.3 B	4.5 (9)	11.0 (22)	19.0 (38)	24.5 (49)
15.6 A	1.0 (2)	2.5 (5)	5.5 (11)	10.0 (20)
15.6 B	0.5 (1)	1.0 (2)	2.0 (4)	4.5 (9)
0.0 A	1.0 (2)	2.0 (4)	3.0 (6)	4.5 (9)
0.0 B	3.5 (7)	3.5 (7)	3.5 (7)	5.5 (11)

Time (Hours)	LC50	95% C.I.	Slope	R^2
24	444	21-1,585	1.53650	0.845
48	94	57-170	2.38034	0.880
96	44	33-58	4.65117	0.961
168	32	24-43	6.17148	0.956

Table 4. Acute toxicity of cadmium to 20-day-old striped bass (Test B).

	Response - Cumulative % Mortality (Actual Mortality)			
Dose Cadmium (µg/l)	Time (hours) 24	48	96	168
32 A	100 (20)	-	-	-
32 B	100 (20)	-	-	-
16 A	85 (17)	85 (17)	95 (19)	100 (20)
16 B	80 (16)	85 (17)	90 (18)	100 (20)
8 A	30 (6)	30 (6)	30 (6)	30 (6)
8 B	25 (5)	30 (6)	30 (6)	40 (8)
4 A	0 (0)	10 (2)	10 (2)	15 (3)
4 B	5 (1)	5 (1)	10 (2)	10 (2)
2 A	5 (1)	5 (1)	10 (2)	10 (2)
2 B	0 (0)	0 (0)	5 (1)	15 (3)
0 A	5 (1)	5 (1)	5 (1)	5 (1)
0 B	0 (0)	5 (1)	5 (1)	5 (1)

Time (Hours)	LC50	95% C.I.	Slope	R^2
24	9	5-17	5.78015	0.848
48	8	5-13	5.32837	0.895
96	6	4-11	4.53941	0.878
168	5	1-13	5.39979	0.715

Table 5. Acute toxicity of cadmium to 128-day-old striped bass (Test C).

Dose Cadmium (µg/l)	Response - Cumulative % Mortality (Actual Mortality) Time (hours) 24	48	96	120
20,000 A	20 (2)	90 (9)	100 (10)	-
20,000 B[a]	0 (0)	80 (8)	90 (9)	90 (9)
10,000 A	0 (0)	60 (6)	90 (9)	90 (9)
10,000 B	40 (4)	80 (8)	100 (10)	-
5,000 A	20 (2)	60 (6)	90 (9)	100 (10)
5,000 B	30 (3)	40 (4)	80 (8)	100 (10)
2,500 A[b]	0 (0)	40 (4)	70 (7)	90 (9)
2,500 B	0 (0)	30 (3)	70 (7)	90 (9)
1,250 A	0 (0)	0 (0)	60 (6)	80 (8)
1,250 B	0 (0)	20 (2)	50 (5)	90 (9)
0 A	0 (0)	0 (0)	0 (0)	0 (0)
0 B[c]	0 (0)	0 (0)	0 (0)	0 (0)

[a] Chemical analysis of test solution = 20,400 µg/l total cadmium.

[b] Chemical analysis of test solution = 2,480 µg/l total cadmium.

[c] Chemical analysis of diluent water = 0.11 µg/l total cadmium.

Time (Hours)	LC50	95% C.I.	Slope	R^2
24	*	*	*	
48	6,173	1,799-27,643	2.62100	0.632
96	1,010	192-1,824	1.43483	0.546
120	922	69-1,880	4.88323	0.799

*Data inadequate to calculate an LC50.

the amount of variation in mortality accounted for by dosage (R^2) in probit analyses conducted to estimate LC50's (Tables 3 and 4). Confidence intervals for 96 and 168-hour LC50 estimates for copper were about ± 30% of the estimate (Table 3), whereas equivalent confidence intervals for cadmium exceeded ± 100% of the LC50 estimate (Table 3).

Cadmium acute toxicity (96-hour LC50) to juvenile striped bass was more than three orders of magnitude lower than the toxicity to larvae (Compare Tables 4 and 5). However, juvenile mortality as not consistently proportional to total cadmium concentration particularly during the last 48 hours of the acute test. This generated the large confidence intervals for LC50 estimates and the relatively low R^2 values from probit analysis of mortality data (Table 5).

Despite large differences in acute toxicity, cadmium and copper caused similar effects on the behavior of striped bass exposed to acutely lethal concentrations. The activity level of treated fry declined with increasing dosage and length of exposure to either metal. Affected fish became progressively lethargic and were eventually immobilized on the bottom of the test container 12-24 hours prior to the onset of morbidity. In some cases, treated fish began swimming in a disoriented fashion, often in upward vertical spirals, and typically continued this activity as intermittent attempts at rising off the bottom before becoming completely immobilized.

Acutely toxic levels of cadmium induced apparent hyperactivity among exposed juveniles in contrast to the behavioral effects on larvae. Treated fish reacted to any type of disturbance by swimming frantically about the test container. Such episodes occasionally preceded a loss of equilibrium among several fish, which also exhibited flared gill covers in an apparent state of tetany. Most of these fish regained equilibrium and swimming ability within a matter of minutes, although several treated juveniles in the cadmium acute test died shortly after losing equilibrium. These mortalities all occurred

in test containers receiving one of the two highest cadmium concentrations (20 or 10 mg Cd^{++}/l).

Results of acute toxicity tests with copper and cadmium from 1985 are summarized in Tables 6 and 7, respectively. Lethal effects of copper on striped bass larvae were most pronounced at low salinity (<0.1%.) and for fish at 17 days post-hatch (Table 6). LC50 estimates for 17-day-old fish tested in 1985 were similar to those for 14-day-old larvae tested in 1984 (Table 3). Copper lethality for striped bass larvae was a consistent dose-response phenomenon as indicated by the relatively large values of R^2 from 96-hour LC50 estimation (Tables 3 and 6). Behavioral effects of copper on striped bass in 1985 tests were very similar to those described from 1984 studies, with progressive lethargy preceding immobilization and death as a common response.

The acute toxicity of cadmium was also highest at low salinity and for striped bass larvae at 17 days post-hatch (Table 7). However, acute toxicity in 1985 static tests was considerably lower (larger LC50 estimates) than that from 1984 flow-through tests with 20-day-old larvae (Table 4). This may be due to: 1) an increase in sensitivity to heavy metals with increasing age for striped bass larvae between 10 and 20 days post-hatch; 2) differences in the sensitivity to toxicants between groups of larvae produced from different parents under varied spawning conditions in different years; 3) differences in the toxicity of cadmium or other factors between static and flow-through tests; and 4) differences in the proportion of anatomically abnormal (uninflated swimbladder, etc.) or physiologically abnormal larvae inadvertently loaded into test containers between different tests. Variation in the lethal response to cadmium is likely a reflection of the biological variability within and between groups of artifically produced striped bass larvae.

Cadmium acute toxicity in 1984 and 1985 testing was more variable as a dose-response phenomenon than the toxicity of copper. LC50 estimates for cadmium had lower R^2 values than estimates for copper indicating a more variable proportionality

Table 6. Acute toxicity of copper (µg/l) to striped bass larvae tested in 1985 at different ages and at various salinities.

Fish Age Days[a]	Salinity ‰	LC50 (95% C.I.) Exposure Time (Hours) 24	48	96	Slope[b]	R^2
3	<0.1	326 (185-530)	152 (129-173)	36 (18-54)	1.69831	0.975
3	1	738 (366-1,889)	465 (339-686)	234 (166-311)	2.02638	0.982
9	5	1,116 (567-1,788)	538 (305-1,011)	463 (321-690)	2.67622	0.941
10	2	697 (337-2,860)	554 (318-1,539)	218 (142-316)	1.41117	0.969
11	1	1.600 (441-7,047)	359 (188-792)	296 (214-423)	2.54592	0.770
17	2	222 (200-251)	160 (158-161)	79 (57-103)	1.74401	0.989

[a] Days after hatching.

[b] Slope and R^2 values are reported only from the 96 hour LC50 calculation.

Table 7. Acute toxicity of cadmium (µg/l) to striped bass larvae tested in 1985 at different ages and at various salinities.

Fish Age Days[a]	Salinity %.	LC50 (95% C.I.) Exposure Time (Hours) 24	48	96	Slope[b]	R^2
3	<0.1	875 (502-1,227)	371 (202-834)	41 (7-89)	1.36966	0.847
3	1	*	158 (71-372)	35 (16-55)	1.28688	0.928
9	5	>50(*)	>50(*)	>50(*)		
10	2	*	748 (242-1,445)	500 (358-806)	1.40119	0.909
11	1	*	779 (478-1,599)	720 (558-1,128)	2.06109	0.836
17	2	1,227 (953-2,545)	769 (534-1,004)	84 (45-125)	0.89401	0.909

[a] Days after hatching.

[b] Slope and R^2 values are reported only from the 96 hour LC50 calculation.

*Data inadequate for necessary calculation.

between cadmium concentration and larval mortality (Compare Table 3 with Tables 4 and 5; compare Tables 6 and Table 7). The behavior of striped bass larvae exposed to lethal cadmium levels in 1985 was virtually identical to that observed in 1984 tests with a progressive decline in activity leading to a terminal loss of mobility.

Chronic Toxicity - Copper

Two partial (60-day) chronic toxicity tests were conducted with young striped bass exposed to copper. Chronic Toxicity Test #1 started with 10 day post-hatch larvae (Sacramento-San Joaquin stock) subjected to the range of copper concentrations indicated in Table 8. Background water quality during this test included the following average and range of the conditions monitored: Temperature - 19 °C (18-20 °C), ammonia - 0.01 mg/l, total hardness - 235 mg/l (232-241), and salinity - 4.9 %. (4.2-5.3). Each test container was stocked with 200 fish.

Soon after the beginning of Test #1 it became apparent that the fish in each test container (diluter aquarium) included two distinct groups: those with an inflated swimbladder and those lacking an inflated swimbladder. The proportion of inflated fish varied between tanks (Table 8) but approximated 0.1 for most aquaria. As a result of this inadvertent difference in test fish, mortality and growth during the experiment were determined separately for the two groups to provide for comparison of results.

Mortality during the first 15 days of Test #1 was similar between inflated and uninflated striped bass larvae (Table 8). In addition, the mortality of the two fish groups combined was proportional to copper concentration as indicated by a significant ($P<0.001$) linear regression of cumulative percent mortality on copper concentration ($Y = 4.171 + 0.196X$, $R^2 = 0.685$; where Y = cumulative percent mortality and X = copper concentration). Mean cumulative mortality at 50 g Cu^{++}/l was also significantly higher ($P<0.01$) than that among control fish (Table 8).

Table 8. Cumulative percent mortality of striped bass during exposure to copper in Chronic Toxicity Test #1.

Test Time (d)[2]	Fish Age (d)	Test[1] Fish	Copper Concentration (µg/l) 50 A	50 B	25 A	25 B	12.5 A	12.5 B	6.3 A	6.3 B	3.1 A	3.1 B	0.0 A	0.0 B
15	25	I	18	7	10	0	0	17	0	11	4	9	0	0
		U	14	16	9	5	8	3	12	7	5	2	3	4
		Comb	15	15	9	5	8	5	11	7	5	3	3	4
		Mean	15.0[a]		7.0[ab]		6.0[b]		9.0[ab]		4.0[b]		3.0[b]	
30	40	I	74	86	33	17	19	33	0	26	13	18	5	12
		U	30	32	18	14	18	9	15	16	12	5	11	10
		Comb	38	40	20	14	18	12	13	17	12	6	10	10
		Mean	39.0[c]		17.0[d]		15.0[d]		15.0[d]		9.0[d]		10.0[d]	
48	58	I	80	86	38	17	19	33	0	26	13	23	5	12
		U	43	42	40	33	43	45	48	38	29	28	49	38
		Comb	50	48	40	32	40	43	43	37	27	27	44	35
		Mean	49.0[e]		36.0[ef]		42.0[ef]		40.0[ef]		27.0[f]		40.0[ef]	
Total (I)			34	29	21	12	21	24	20	19	24	22	20	25
Total (U)			166	171	179	188	179	176	180	181	176	178	180	175
Ratio I:U			.21	.17	.12	.06	.12	.14	.11	.10	.14	.12	.11	.14

[1] Test fish in each diluter aquarium included individuals with an inflated (I) swimbladder and individuals which failed to inflate (U). Mortality was determined separately for these two groups, and expressed as a percent of the total individuals in the respective group. Percent mortality within each diluter aquarium is indicated as "Combined" (Comb), with the two (Comb) percentages averaged as a mean percent mortality for each copper concentration. Means with one or more superscripts in common, are not significantly different (P>0.05) from Duncan's new multiple range test.

[2] (d)=days

After 30 days exposure, combined mortality of striped bass larvae was still proportional to copper concentration ($Y = 8.786 + 0.548X$, $R^2 = 0.865$) and combined mortality at the highest concentration was significantly greater ($P<0.001$) than at all other concentrations and the control (Table 8). However, the mortality of inflated fish at 50 g Cu^{++}/l was more than twice that of uninflated fish in the same aquaria by day 30 of the test.

Toxicity Test #1 was terminated after 48 days because cumulative mortality of control fish was approaching that at the highest copper dosage. Increased control mortality was part of a general die-off among uninflated fish in all test containers which coincided with high mortality of uninflated fish in the holding tank. Mortality of uninflated fish between days 30 and 48 of the experiment is the major reason for the lack of significant difference among most of the means for combined mortality at the various copper concentrations (Table 8).

Copper concentrations measured in water samples from Chronic Test #1 were generally close to the expected concentrations, with the exception of the first water sample collected on 5/18/84 (Table 9). This is also the only sample for which a paired "t" test indicated any significant difference ($P<0.05$) in the measured copper concentration between replications (A and B). The cause for these anomalous results is unknown because proportional diluter operation was trouble-free during this entire test. For most samples, measured copper concentration exceeded nominal levels by several $\mu g/l$ (Table 9).

As an alternative method of analyzing the mortality data from Test #1, a series of linear regressions of cumulative percent mortality (combined) on elapsed time (days) were performed for each copper concentration and control over three time intervals: 0-15, 0-30, and 0-48 days, respectively. The resulting slopes (regression coefficients) were tested for significant differences by the Simultaneous Test Procedure of Sokal and Rohlf (1969, Section 14.9), and are presented in Table 10. As expected, the slope of cumulative mortality regressed on

Table 9. Copper concentrations (µg/l) measured in water samples from Chronic Toxicity Test #'s 1 and 3, including recovery of copper from quality control reference materials.

Analyzed Copper (µg/l)

Nominal Dose (µg/l)	Test #1 Sample Date 5/18	6/12	6/20	6/29	Nominal Dose	Test #3 Sample Date 7/15	7/30	8/14	8/29	9/13
50 A	62.6	53.8	52.2	51.7	100 A	107.8	109.2	111.5	107.7	110.0
50 B	62.1	53.8	51.6	52.3	100 B	106.9	112.0	111.1	107.7	108.3
25 A	28.3	25.0	26.0	25.0	50 A	52.0	52.5	52.6	58.7	51.3
25 B	27.8	22.3	24.8	25.5	50 B	50.6	53.4	53.9	56.1	52.1
12.5 A	22.0	17.5	15.4	14.3	25 A	28.0	28.9	29.5	30.2	28.5
12.5 B	14.7	14.8	14.2	13.7	25 B	28.5	29.4	29.0	28.9	26.8
6.3 A	12.8	10.0	8.4	8.1	12.5 A	17.4	17.4	16.8	18.7	17.0
6.3 B	17.6	7.8	8.4	8.1	12.5 B	14.6	14.6	14.7	16.1	13.8
3.1 A	10.4	5.2	5.5	4.9	6.3 A	8.6	7.2	8.0	9.7	8.1
3.1 B	9.4	7.3	4.9	5.6	6.3 B	8.6	7.7	8.4	13.1	8.5
0.0 A	4.6	<LOD[a]	<LOD	<LOD	0.0 A	<LOD	<LOD	<LOD	5.0	<LOD
0.0 B	4.6	<LOD	<LOD	<LOD	0.0 B	<LOD	<LOD	<LOD	5.9	<LOD
Wet Lab H_2O	<LOD	<LOD	<LOD	<LOD	Wet Lab H_2O	<LOD	<LOD	<LOD	<LOD	<LOD

Quality Control for Copper

Reference Material	N	X	S.D.	Certified Conc. (µg/l)
NBS H_2O	7	19.3 (µg/l)	± 1.0	18 ± 2
Spike	2	100.0%	± 0.0	10
Spike	12	98.4%	± 1.7	20
Spike	4	102.1%	± 1.7	40

[a] LOD = Limit of Detection = 3.9 µg/l.

.ole 10. Comparison of slopes among linear regressions of cumulative percent mortality on time (days) within each copper concentration of Chronic Toxicity Test #1.

Test Time (d)[2]	Fish Age (d)	Regr. Stat.	Copper Concentration (µg/l) 50	25	12.5	6.3	3.1	0.0
15	25	Slope[1]	0.804[a]	0.403[c]	0.401[c]	0.580[b]	0.262[d]	0.172[d]
		95% CI	.699-.908	.308-.497	.275-.527	.449-.712	.182-.341	.129-.215
		R^2	0.898	0.855	0.601	0.744	0.788	0.705
30	40	Slope	1.095[e]	0.526[f]	0.479[f]	0.514[f]	0.309[g]	0.296[g]
		95% CI	1.01-1.18	.467-.584	.422-.537	.470-.558	.266-.352	.265-.326
		R^2	0.912	0.846	0.829	0.903	0.781	0.869
48	58	Slope	1.167[h]	0.799[i]	0.912[i]	0.843[i]	0.577[j]	0.897[i]
		95% CI	1.12-1.22	.739-.858	.836-.989	.769-.918	.523-.631	.801-.993
		R^2	0.962	0.884	0.856	0.845	0.828	0.787

[1] Slopes with one or more superscripts in common, are not significantly different (P>0.05) from the Simultaneous Test Procedure of Sokal and Rohlf (1969, Section 14.9),

elapsed time increased within each copper concentration, with increasing exposure time (compare slopes within the columns of Table 10). In addition, the pattern of significant differences in slopes among copper concentrations for each time period (compare slopes across the rows of Table 10), is similar to the pattern of differences between means of cumulative mortality in Table 8. A surprising result of this analysis is the relatively large amount of variation in cumulative mortality explained by linear regression on elapsed time. This is indicated from the relatively large coefficients of determination (R^2) in Table 10 and the highly significant difference ($P<0.01$) from zero for all of the slopes in Table 10, determined from regression tests of significance (Sokal and Rohlf 1969, Section 14.6).

The second partial chronic test with copper was labeled Chronic Toxicity Test #3 and was initiated with 75 day-old (post-hatch) juveniles. These fish were taken from the same lot of Sacramento-San Joaquin striped bass used to supply fish for Test #1. Water quality during this test included the following average and range of conditions: Temperature - 19 °C (18-20 °C), dissolved oxygen - 5.9 mg/l (4.2-6.5 mg/l), pH - 7.4 (7.0-7.5), ammonia - <0.01 mg/l, total hardness - 238 mg/l (233-244) and salinity - 5.1%. (4.7-5.9%.). Nominal copper concentrations for this test ranged from 100 µg/l to 6.3 µg/l (Table 11).

Levels of copper analyzed from water samples taken during Toxicity Test #3 were consistently close to but several µg/l higher than nominal concentrations (Table 9). A paired "t" test of these data indicated no significant differences ($P>0.05$) in copper content between replications. Copper concentrations of 2 - 4.6 µg/l in control water samples were due to background copper from Instant Ocean® salts added to the water plus background copper in the laboratory water supply (Table 9).

Significant mortality during Test #3 occurred only at the highest copper dosage (100 µg/l) during the first 15 days of the test. Mortality at other concentrations and the controls was very low or zero (Table 11). Copper concentrations which were lethal to striped bass larvae in Test #1, had no effect on

Table 11. Cumulative percent mortality of juvenile striped bass exposed to copper during Chronic Toxicity Test #3.

Test Time Days	Fish Age Days	Copper Concentration (µg/l) 100 A	100 B	50 A	50 B	25 A	25 B	12.5 A	12.5 B	6.3 A	6.3 B	0.0 A	0.0 B
15	90	40	20	0	0	0	0	0	0	0	0	0	0
	Mean	30		0		0		0		0		0	
30	105	40	20	0	0	0	0	0	0	0	0	0	0
	Mean	30		0		0		0		0		0	
	135	40	28	0	0	0	5.6	0	5.6	0	5.6	0	5.6
	Mean	34		0		3		3		3		3	

juvenile survival despite the longer period of exposure of juveniles in Test #3 (Compare Tables 8 and 11). The pattern of juvenile mortality at 100 µg/l in Test #3 indicates an apparent period of acclimation to elevated copper concentrations during which some mortality occurs but after which surviving fish are more resistant to lethal effects.

Copper - Growth

Growth of striped bass in Chronic Toxicity Test #1 was very different between inflated and uninflated fish groups over all copper concentrations and the controls. After 15 days, the mean weight of inflated fish ranged from 1.3 to 2.2 times that of uninflated fry in the various test containers (Table 12). At 30 days, this range declined (1.1 to 1.7) but after 48 days inflated fish mean weight averaged 2.2 to 6.5 times the mean weight of uninflated fish. Part of this increased difference in weight between the two groups is due to the accelerated mortality of uninflated fish during the last 18 days of the toxicity test (Table 8), particularly the larger uninflated fish in each test container.

Differences in mean total length between inflated and uninflated fish were less pronounced than differences in mean weight (Table 12). In general, the length of striped bass was not as good an indicator of growth differences as total weight because small changes in length are associated with large changes in weight during this life history stage.

Copper concentrations in Test #1 had little effect on the growth of striped bass with the exception of uninflated fish after 15 days (Table 12). All other comparisons of mean weight between fish exposed to copper and control fish indicated no significant differences ($P>0.05$). As a result, statistical comparisons of mean total length across copper concentrations and the controls were not performed due to the small differences in mean lengths within inflated and uninflated groups at each sampling point (15, 30, and 48 days) during the test (Table 12).

Copper concentrations tested in Toxicity Test #3 had little effect on the growth of juvenile striped bass except at the

ble 12. Mean total length (mm) and mean weight (mg) of striped bass exposed to copper during Chronic Toxicity Test #1.

Test Time (d)	Age (d)	Fish Test[1]		Copper Concentration (µg/l) 50 A	50 B	25 A	25 B	12.5 A	12.5 B	6.3 A	6.3 B	3.1 A	3.1 B	0.0 A	0.0 B
15	25	Inflated	L[2]	18	18	17	19	19	17	19	18	20	19	19	19
			W[2]	52[a]	48[a]	47[a]	50[a]	53[a]	46[a]	49[a]	46[a]	55[a]	51[a]	53[a]	52[a]
		Uninflat.	L	14	15	15	16	16	17	15	15	15	16	15	16
			W	24[d]	25[d]	27[cd]	35[bcd]	33[bcd]	42[b]	29[cd]	30[cd]	33[bcd]	39[bc]	33[bcd]	35[bcd]
		Ratio	L[3]	1.3	1.2	1.1	1.2	1.2	1.0	1.2	1.2	1.3	1.2	1.2	1.2
		Ratio	W[4]	2.2	2.0	1.8	1.4	1.6	1.1	1.7	1.5	1.7	1.3	1.6	1.5
30	40	Inflated	L	24	26	26	27	26	27	26	25	25	27	27	25
			W	127[e]	130[e]	136[e]	136[e]	133[e]	139[e]	132[e]	132[e]	130[e]	146[e]	135[e]	129[e]
		Uninflat.	L	22	20	21	21	21	24	22	20	22	23	22	23
			W	86[f]	80[f]	88[f]	90[f]	88[f]	123[f]	100[f]	78[f]	105[f]	111[f]	85[f]	119[f]
		Ratio	L	1.1	1.3	1.2	1.3	1.2	1.1	1.2	1.3	1.1	1.1	1.2	1.1
		Ratio	W	1.5	1.6	1.5	1.5	1.5	1.1	1.3	1.7	1.2	1.3	1.6	1.1
48	58	Inflated	L	43	41	43	41	45	43	41	44	42	44	41	42
			W	769[g]	654[g]	766[g]	697[g]	933[g]	720[g]	722[g]	879[g]	656[g]	797[g]	642[g]	700[g]
		Uninflat.	L	29	32	30	29	28	32	30	27	29	29	29	28
			W	205[h]	294[hi]	229[hi]	222[hi]	194[hi]	268[hi]	225[hi]	134[i]	200[hi]	206[hi]	226[hi]	190[hi]
		Ratio	L	1.5	1.3	1.4	1.4	1.6	1.3	1.4	1.7	1.4	1.5	1.4	1.5
		Ratio	W	3.7	2.2	3.3	3.1	4.8	2.7	3.2	6.5	3.3	3.9	2.8	3.7

[1] Length and weight were determined separately for fish with an inflated swimbladder and for individuals which failed to inflate, based on a sample of four (4) fish of each group from each diluter aquarium at each sampling time.
[2] L = mean total length (mm); W = mean weight (mg)
[3] Ratio L = mean total length of inflated fish sample divided by mean length of uninflated sample.
[4] Ratio W = inflated fish mean weight divided by uninflated fish mean weight.

highest concentration (Table 13). As in Test #1 with larvae, the mean weight of juveniles subjected to the highest copper concentration (100 µg/l), was significantly less at 15 days ($P<0.001$) than that of fish at all other concentrations and the controls (Table 13). However, fish growth at this copper level increased during the second 15 days of exposure and equaled or exceeded that of all other treated and control groups by day 30.

After 60 days, there was no significant difference ($P>0.05$) in mean weight between any copper treatment and at least one of the two control groups (Table 13). In addition, linear regression of fish weight on copper concentration at day 30 and day 60 of the test, indicated no significant ($P>0.05$) linear relationship between weight and dosage.

As a method to characterize the growth of striped bass tested with copper, a series of weight (mg) on total length (mm) regressions were performed for all of the fish in both toxicity tests (#1 and #3) at each sampling time (15, 30, and 48 or 60 days). The results of this analysis are presented in Table 14. For all groups of fish tested, there was a significant ($P<0.01$) linear relationship between weight and total length, as expected. This is indicated in Table 14 by the relatively large values of R^2, the proportion of variation in weight explained by changes in total length. Comparison of the slopes from weight on length regressions indicated a general increase in slope (weight per unit length) over time for striped bass larvae at all three sampling times but a non-significant ($P>0.05$) change in slope after 30 days for juvenile striped bass (Table 14). These weight on length data do not deal with growth in relation to copper concentration but are provided as an index of the length-weight relationships among the aggregate of test fish used to study the effects of copper. However, a separate weight on length regression analysis for each copper concentration in Test #'s 1 and 3 demonstrated significant differences in slope between concentrations. The pattern of these differences was virtually identical to the pattern of significant differences in mean weights given in Tables 12 and 13, respectively.

Table 13. Mean total length (mm) and mean weight (g) of striped bass juveniles exposed to copper during Chronic Toxicity Test #3. Mean weights with one or more superscript letters in common are not significantly different (P>0.05) from Duncan's new multiple range test.

Test Time (d)	Fish Age (d)	Data	Copper Concentration (µg/l) 100 A	100 B	50 A	50 B	25 A	25 B	12.5 A	12.5 B	6.3 A	6.3 B	0.0 A	0.0 B
		L[1]	57	60	66	65	66	66	67	68	69	67	71	70
15	90	N	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
		W[1]	1.8[a]	2.2[a]	3.1[bc]	2.9[bc]	2.8[b]	3.0[bc]	3.1[bc]	3.2[bc]	3.3[bc]	3.1[bc]	3.6[c]	3.4[bc]
		L	73	71	70	72	73	73	72	74	69	73	70	70
30	105	N	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
		W	4.4[e]	3.9[d]	3.9[d]	4.2[e]	4.6[e]	4.6[e]	4.3[e]	4.7[e]	3.6[d]	4.7[e]	3.8[d]	3.7[d]
		L	85	82	85	88	85	90	87	89	81	89	75	87
60	135	N[2]	(10)	(13)	(18)	(18)	(17)	(17)	(18)	(17)	(18)	(17)	(18)	(17)
		W	6.6[gh]	5.7[fg]	6.3[gh]	6.7[gh]	6.5[gh]	7.5[h]	6.8[gh]	7.3[gh]	5.6[fg]	6.8[gh]	4.5[g]	6.7[gh]

[1] L = mean total length (mm); W = mean weight (g).

[2] Sample size varied at the conclusion of the test due to differences in survival between diluter tanks.

Table 14. Comparison of slopes among linear regressions of weight (mg) on length (mm) for striped bass in chronic toxicity tests with copper.

Toxicity Test #	Striped Bass	Regression Statistic	Exposure Period (Days) 15	30	End[1]
1	Inflated Larvae	Slope[2]	3.52[a]	4.23[a]	55.07[b]
		(95% C.I.)	(2.48-4.56)	(3.21-5.24)	(44.22-65.93)
		R^2	0.72	0.69	0.76
	Uninflated Larvae	Slope	6.27[c]	11.13[d]	21.20[e]
		(95% C.I.)	(5.32-7.21)	(9.66-12.61)	(18.86-23.53)
		R^2	0.80	0.83	0.88
3	Juvenile	Slope	139.19[f]	230.59[g]	220.23[g]
		(95% C.I.)	(131.0-147.4)	(214.9-246.3)	(213.1-227.4)
		R^2	0.92	0.90	0.95

[1] Toxicity Test #1 ended after 48 days and Toxicity Test #3 concluded after 60 days.

[2] Slopes with one or more superscript letters in common are not significantly ($P>0.05$) different.

Cadmium - Mortality and Growth

Chronic toxicity of cadmium to young striped bass was studied in a 60 day partial chronic test (Chronic Toxicity Test #2) initiated with 45 day-old juveniles. These fish all had an inflated swimbladder and were from the same lot of Sacramento-San Joaquin stock used to supply fish for Chronic Test #'s 1 and 3. A total of 50 fish was stocked into each test container. Water quality during this test included the following average and range of conditions: Temperature - 20 °C (19-21 °C), dissolved oxygen - 5.7 mg/l (4.3-6.0 mg/l), pH - 7.4 (7.2-7.5), ammonia - 0.1 mg/l (0.01-0.25 mg/l), total hardness 240 mg/l (233-243), and salinity - 5.3%. (4.2-6.4%.). Cadmium concentrations tested were 10, 5, 2.5, 1.3, and 0.6 µg/l, plus diluent water controls.

Cadmium concentrations measured from Chronic Test #2 closely approximated expected levels, but were generally above nominal dosage (Table 15). Measured concentrations of cadmium were closer to expected values than measured and expected concentrations of copper (Table 9) because Instant Ocean^{R} and the laboratory water supply contained very little cadmium (Table 15).

Mortality of striped bass juveniles during Chronic Toxicity Test #2 was zero at all cadmium concentrations and the controls. Although several fish in each test container at 10 and 5 µg/l were obviously stressed (darkened and lethargic) during the first 2 days of the test, they recovered to normal color and behavior during the first week of the test.

Growth of striped bass was not adversely affected at any of the cadmium concentrations tested. The mean weight of fish from cadmium treated tanks was not significantly different ($P>0.05$) from that of control fish after 15 and 30 days of exposure (Table 16). After 60 days, however, mean weight in some tanks at the two highest cadmium concentrations was significantly greater ($P<0.001$) than mean weight in either control replicate (Table 16). Regression analysis of weight on total length for all of the fish in Chronic Toxicity Test #2 after 15, 30, and 60 days, produced slopes and 95% confidence intervals of 53.185 ± 2.758, 74.251 ± 4.511, and 119.170 ± 4.049, respectively. These slopes

Table 15. Cadmium concentrations (µg/l) measured in water samples from Chronic Toxicity Test # 2, and recovery of cadmium from quality control reference materials.

Nominal Dose (µg/l)	Analyzed Cadmium (µg/l) Sample Date 6/18	6/30	7/15	7/30	8/14
10 A	11.1	10.6	11.5	12.7	11.1
10 B	11.1	10.9	11.2	13.5	11.2
5 A	5.2	5.2	5.5	7.9	5.7
5 B	5.4	5.5	5.6	8.3	5.7
2.5 A	3.0	3.0	3.0	3.8	2.9
2.5 B	2.5	2.6	2.7	3.3	2.5
1.3 A	1.3	1.5	1.5	2.1	1.5
1.3 B	1.2	1.2	1.3	1.6	1.3
0.6 A	0.6	0.7	0.7	1.1	0.7
0.6 B	0.6	0.7	0.7	1.0	0.8
0.0 A	<LOD[1]	<LOD	<LOD	<LOD	<LOD
0.0 B	<LOD	<LOD	<LOD	<LOD	<LOD
Wet Lab H_2O	<LOD	<LOD	<LOD	<LOD	<LOD

Quality Control for Cadmium

Reference Material	N	X	S.D.	Certified Conc. (µg/l)
NBS H_2O	6	10.5 (µg/l)	± 0.4	10 ± 1
Spike	12	101.6%	± 3.4	1

[1] LOD = Limit of Detection = 0.2 µg/l.

. ble 16. Mean total length (mm) and mean weight (g) of striped bass juveniles exposed to cadmium in Chronic Toxicity Test #2. Mean weights with one or more superscript letters in common are not significantly different (P>0.05) from Duncan's new multiple range test.

Test Time (d)	Fish Age (d)	Data	Cadmium Concentration (µg/l) 10 A	10 B	5 A	5 B	2.5 A	2.5 B	1.25 A	1.25 B	0.6 A	0.6 B	0.0 A	0.0 B
		L[1]	41	41	38	40	41	41	41	42	39	40	41	40
15	60	N	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
		W[1]	.67[a]	.64[a]	.56[a]	.65[a]	.67[a]	.70[a]	.70[a]	.63[a]	.59[a]	.61[a]	.64[a]	.64[a]
		L	52	49	53	50	51	53	47	47	50	50	46	51
30	75	N	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
		W	1.4[b]	1.1[c]	1.4[b]	1.1[c]	1.2[b]	1.2[b]	1.0[c]	.98[c]	1.2[b]	1.2[bc]	.95[c]	1.3[b]
		L	68	65	68	68	67	66	66	66	65	67	62	64
30	105	N[2]	(44)	(42)	(40)	(44)	(43)	(44)	(44)	(44)	(43)	(44)	(42)	(43)
		W	2.7[d]	2.1[e]	2.7[d]	2.8[d]	2.5[de]	2.4[de]	2.4[e]	2.4[de]	2.3[e]	2.5[de]	2.1[e]	2.2[e]

[1] L = mean total length (mm); W = mean weight (g).

[2] Sample size varied at the conclusion of the test due to differences in survival between diluter tanks.

were all significantly different ($P<0.01$) and indicated a trend of increasing weight per unit length among striped bass juveniles similar in pattern but greater in magnitude than weight per unit length increases among younger (smaller) fish in Chronic Test #1 (Table 14).

Tissue Residue - Bioconcentration

Whole body residues of copper in striped bass from Chronic Toxicity Test #1 were proportional to copper concentrations in test water (Table 17). This linear relationship was significant ($P<0.001$), as determined by regression of tissue residue on water concentration which produced the least squares fitted equation: $C_F = 0.1296 + 0.0613\ C_W$; $R^2 = 0.97$. However, one-way analysis of variance indicated that only the mean residue in fish from the highest test water concentration (55 µg/l) was significantly different ($P<0.001$) from residues in control fish. Copper residues in fish exposed to 55 µg/l in water, were three times higher than the copper content of fish treated at 25 µg/l (Table 17).

BCF values for copper in early juvenile striped bass were variable, with minimum BCF at intermediate copper concentrations in water, and maximum values in the control (Table 17). Bioconcentration of copper at low levels in the control water is indicative of uptake and conservation of an essential element.

Copper residues in older striped bass juveniles from Chronic Toxicity Test #3 were linearly related to copper concentrations in water (Table 18). The least squares regression equation for this relationship was $C_F = -0.0540 + 0.0394\ CW$; $R^2 = 0.84$ and the regression coefficient (slope) significantly greater ($P<0.01$) than zero. Striped bass subjected to the highest metal concentration (109 µg/l) were the only fish with whole body residues significantly ($P<0.001$) greater than control fish residues. Fish surviving exposure to 109 µg/l had four times the body burden of copper among survivors at 50 µg/l (Table 18). Control fish had copper concentrations very similar to those present in untreated fish from Toxicity Test #1 (Tables 17 and 18).

.ble 17. Copper concentrations in test water compared with whole body residues in striped bass exposed for 48 days during Chronic Toxicity Test #1. Calculated bioconcentration factors (BCF) are also included.

Water				Tissue				
Nominal Dose (µg/l)		Sample Size	Mean[1] Conc. (µg/l)	Composite Sample Size	Fish Mean Wt. (mg)	Percent Moisture	Copper[2] Residue (µg/g)	BCF
50	A	4	55.1	81	316	80.0	3.83	70
50	B	4	55.0	95	307	79.9	3.46	63
25	A	4	26.1	111	367	79.7	1.34[3]	51
25	B	4	25.1	111	314	79.9	1.66	66
12.5	A	4	17.3	90	352	79.4	0.91	53
12.5	B	4	14.4	103	361	80.0	0.84	58
6.3	A	4	9.8	104	336	79.5	0.70	71
6.3	B	4	10.5	132	261	81.1	0.69	66
1	A	4	6.5	123	275	80.4	0.60	92
1	B	4	6.8	123	324	80.6	0.67[3]	99
0.0	A	4	<LOD	90	319	79.4	0.59	184
0.0	B	4	<LOD	121	336	80.6	0.53	165

Quality Control for Copper

Reference Material	N	X	S.D.	Certified Conc. (µg/g)
NBS Oysters	3	60.1 (µg/g)	± 0.3	63.0 ± 3.5
NBS Bovine Liver	3	183 (µg/g)	± 1.0	193 ± 10
Reagent Blank	8	30.6 (µg/l)	± 19.5	----
Tissue Spike	2	93.6%	± 1.2	3.0
Tissue Spike	2	98.3%	± 4.1	5.0
Analytical STD.	11	96.6%	± 2.7	

Water LOD = Limit of Detection = 3.9 µg/l
Tissue LOD = Limit of Detection = 0.27 µg/g

[1] Average of copper concentrations reported in Table 9.

[2] Residues are based on wet weight of fish.

[3] Mean of triplicate analyses. S.D. in 25A = 0.02µg/g; S.D. in 3.1B =.04µg/g.

Table 18. Copper concentrations in test water compared with whole body residues in juvenile striped bass exposed for 60 days in Chronic Toxicity Test #3. Calculated bioconcentration factors (BCF) and copper recovery from spiked fish are also included.

Water				Tissue				
Nominal Dose (µg/l)		Sample Size	Mean[1] Conc. (µg/l)	Composite Sample Size	Fish Mean Wt. (mg)	Percent Moisture	Copper[2] Residue (µg/g)	BCF
100	A	5	109.2	4	5,768	72.1	4.39	40
100	B	5	109.2	5	4,306	75.7	5.31	49
50	A	5	53.4	10	4,992	72.7	1.11	21
50	B	5	53.2	10	6,974	72.1	0.85[3]	16
25	A	5	29.0	9	6,104	70.5	0.61	21
25	B	5	28.5	9	7,587	71.7	0.59	21
12.5	A	5	17.5	10	6,753	70.8	0.62	35
12.5	B	5	14.8	9	7,647	70.9	0.60	41
6.3	A	5	8.3	10	5,226	71.3	0.63	76
6.3	B	5	9.3	9	7,489	71.5	0.66[3]	71
0.0	A	5	<LOD	6	4,373	72.2	0.68	227
0.0	B	5	<LOD	5	6,506	71.7	0.59	190

Copper Recovery From Spiked Fish

Fish No.	Total Wt. (mg)	Percent Moisture	Copper Spike (µg)	Copper[2] Residue (µg/g)	Percent Recovery
1	2,360	74.3	4	2.24	94.3
2	3,020	74.3	5	2.11	90.3
3	3,340	74.3	5	2.10	96.5

Water LOD = Limit of Detection = 3.9 µg/l
Tissue LOD = Limit of Detection = 0.27 µg/g

[1] Average of copper concentrations reported in Table 9.

[2] Residues are based on wet weight of fish.

[3] Mean of triplicate analyses. S.D. in 50B = 0.02µg/g; S.D. in 6.3B =.09µg/g

BCF values for older striped bass juveniles from Chronic Test #3 were similar to those for younger fish from Test #1, both in magnitude and relationship to copper dosage. The lowest BCF for older fish occurred at median and near-median copper concentrations in test water (Table 18). This apparent parabolic relation between BCF and copper dosage is likely due to metabolic control of copper as an essential element in striped bass. Sharp increases in body burden at the two highest copper concentrations suggests a homeostatic mechanism for copper regulation, overcome by environmental concentrations of the metal above an equilibrium threshold.

Cadmium accumulation in juveniles from Chronic Toxicity Test #2 closely approximated a direct proportion with cadmium concentration in test water (Table 19). The linear regression equation which best described this proportionality was: $C_F = 0.0130 + 0.0584\ C_W$; $R^2 = 0.97$. The slope of this line (0.0584) was significantly greater ($P<0.001$) than zero indicating a positive linear relationship between body burden and dosage. Mean cadmium residues at the various test concentrations, were all significantly different ($P<0.0001$) from each other and the controls based on analysis of variance and multiple means comparison (Duncan's New Multiple Range) tests.

BCF values were very similar over all cadmium levels and the control (Table 19). This bioconcentration pattern reflected the direct proportionality between test water concentration and accumulated body burden. There were no apparent trends for copper and cadmium residues in exposed striped bass in comparison with the weight of individual fish. However, composite residue samples combined a range of fish sizes, thereby masking potential differences in accumulation related to individual biomass.

Biochemical and Mechanical Indicators

Biochemical characteristics of vertebrae from striped bass exposed to copper, indicated few differences in bone development between control and treated fish (Table 20). However, bone calcium concentration was significantly reduced among fish at all but the lowest copper concentration in comparison with controls.

Table 19. Cadmium concentrations in test water compared with whole body residues in juvenile striped bass exposed for 60 days in Chronic Toxicity Test #2. Calculated bioconcentration factors (BCF) and cadmium recovery from reference standards included.

Water				Tissue				
Nominal Dose (µg/l)		Sample Size	Mean[1] Conc. (µg/l)	Composite Sample Size	Fish Mean Wt. (mg)	Percent Moisture	Copper[2] Residue (µg/g)	BCF
10	A	5	11.4	36	2,619	72.2	0.662	58
10	B	5	11.6	34	2,014	74.2	0.697	60
5	A	5	5.9	32	2,715	71.6	0.348[3]	59
5	B	5	6.1	33	2,727	72.9	0.385	63
2.5	A	5	3.1	35	2,561	72.8	0.204	66
2.5	B	5	2.7	36	2,546	72.9	0.190	70
1.3	A	5	1.6	36	2,376	72.8	0.101	63
1.3	B	5	1.3	36	2,474	72.2	0.095	73
0.6	A	5	0.8	35	2,211	72.7	0.057	71
0.6	B	5	0.8	36	2,506	72.2	0.056[3]	70
0.0	A	5	<LOD	34	2,065	73.0	0.005	50
0.0	B	5	<LOD	35	2,172	73.4	0.008	80

Quality Control for Cadmium

Reference Material	N	X	S.D.	Certified Conc. (ng/g)
NBS Tuna	3	41 (ng/g)	± 6	27 - 98
NBS Bovine Liver	3	259 (ng/g)	± 9	230 - 310
NBS Oysters	3	3402 (ng/g)	± 19	3100 - 3400
Reagent Blank	4	0.53 (ng/g)	± 0.48	---
Analysis Spike	10	94.4%	± 1.8	---
Tissue Spike	2	96.8%	± 2.4	236
Whole Fish Spike	3	97.4%	± 3.1	---

Water LOD = Limit of Detection = 0.2 µg/l
Tissue LOD = Limit of Detection = 2.0 ng/g

[1] Average of cadmium concentrations reported in Table 15.

[2] Residues are based on wet weight of fish.

[3] Mean of triplicate analyses: S.D.in 5A = 0.01ng/g; S.D. in 0.6B =.0005ng/g.

Proline:hydroxyproline ratios at the two highest copper levels were also significantly lower than those at all other test concentrations and the controls. Bone density and mechanical properties could not be determined for striped bass larvae because vertebral elements from these fish were too small for accurate analysis. Biochemical data in Table 20 were all taken from fish with an inflated swimbladder, to remove inflation-related differences in growth as a potential source of variation.

Bone development properties for striped bass juveniles treated with copper were generally greater in magnitude than those for control fish (Table 21). Again, however, proline: hydroxyproline ratios at the two highest copper concentrations were significantly below control fish ratios. Collagen concentration also declined significantly at the highest copper levels. Mechanical strength and density of juvenile vertebrae were broadly proportional to mean fish size regardless of copper concentration. Modulus of elasticity was the only measure of bone structural integrity significantly lower in treated than in control fish (Table 21).

Cadmium concentrations had little effect on biochemical characteristics of vertebrae from exposed striped bass fry (Table 22). Bone density was lowest at the highest cadmium concentration (10 µg/l) although the difference from controls was not statistically significant. Mechanical properties including modulus of elasticity and bone toughness were significantly lower at the highest cadmium dosage. Also, levels of force (g-force/mm^2) necessary to rupture vertebrae decreased with increasing cadmium concentration for the four highest cadmium levels tested. Percent strain and elastic limit were lowest at the highest cadmium concentration but not significantly different from the control values (Table 22). Decreased bone strength (rupture and elastic limit) and energy-absorbing capacity (toughness) after cadmium exposure at 10 µg/l was notable because the fish sampled for bone development testing from this concentration were significantly larger than those from the controls and most other concentrations.

Table 20. Mean biochemical composition of cervical vertebrae compared to the size of striped bass treated with copper for 48 days in Chronic Toxicity Test #1. Asterisks indicate significant difference from control values at **P<0.05 and *P<0.10.

	Nominal Copper Concentration (µg/l)					
Characteristic	0.0	3.1	6.3	12.5	25	50
Biochemical Composition						
Collagen (mg/g bone)	184	188	157	181	177	168
Hydroxyproline (HYD)[1]	65	67	64	62	63	66
Proline (PRO)[1]	84	86	92	83	86	81
PRO:HYD ratio[1]	1.31	1.29	1.36*	1.31	1.27*	1.23**
HYD (mg/g bone)	16	15	15	14	14	15
PRO (mg/g bone)	24	24	23	23	22	23
PRO:HYD ratio (bone)	1.48	1.61	1.57	1.61	1.59	1.56
Calcium (mg/g bone)	221	202	170**	192*	185**	185**
Phosphorus (mg/g bone)	153	159	142	135	153	137
Ca + P/Collagen	1.90	1.95	2.01	1.90	1.90	2.09
Density (mg/cm^3)[2]						
Mechanical Properties[2]						
Fish Size						
Length (mm)	41	43	43	44	42	42
Weight (g)	0.67	0.73	0.80	0.83	0.73	0.71
Number of Fish	8	8	8	8	8	8

[1] Measured as mg/g collagen; ratio of PRO:HYD in collagen.

[2] Small fish size precluded determination.

Table 21. Mean vertebral composition, density, and mechanical properties and mean size of juvenile striped bass exposed to copper for 60 days in Chronic Toxicity Test #3. Asterisks denote significant differences from control values at **P<0.05 and *P<0.10.

	Nominal Copper Concentration (µg/l)					
Characteristic	0.0	6.3	12.5	25	50	100
Biochemical Composition						
Collagen (mg/g bone)	212	216	210	211	196*	196*
Hydroxyproline (HYD)[1]	57	62**	63**	64**	70**	69**
Proline (PRO)[1]	82	88	90	97**	93**	88
PRO:HYD ratio[1]	1.43	1.42	1.43	1.52	1.34*	1.28**
HYD (mg/g bone)	15	15	15	15	15	16**
PRO (mg/g bone)	23	23	23	23	24*	26**
PRO:HYD ratio (bone)	1.51	1.49	1.55	1.56	1.55	1.59**
Calcium (mg/g bone)	198	199	183	179	199	195
Phosphorus (mg/g bone)	115	119	119	114	121*	120
Ca + P/Collagen	1.49	1.47	1.42	1.40	1.65*	1.62
Density (mg/cm^3)	243	253	260	294**	287**	284**
Mechanical Properties						
Rupture ($g\text{-force}/mm^2$)	454	499**	462	540**	496*	457
Elastic Limit ($g\text{-force}/mm^2$)	422	456	418	504**	469*	425
% Strain	4.8	5.3**	5.2**	5.0	5.6**	5.2**
Modulus of Elasticity ($\frac{kg\text{-force}}{mm^2}$)	11.97	12.02	11.18	13.12*	11.25	10.87*
Toughness ($g\text{-}mm/m^3$)	12.8	15.7**	14.3	15.8**	16.6**	14.1
Fish Size						
Length (mm)	84	87	90	90	89	87
Weight (g)	6.26	7.01	7.17	7.35	7.13	6.57
Number of Fish	8	8	8	8	8	8

[1] Measured as mg/g collagen; ratio of PRO:HYD in collagen.

Table 22. Characteristics of vertebral bone development and mean size of striped bass exposed to sublethal cadmium levels for 60 days in Chronic Toxicity Test #2. Asterisks designate significant differences from control values at **P<0.05 and *P<0.10.

	Nominal Cadmium Concentration (µg/l)					
Characteristic	0.0	0.6	1.25	2.5	5	10
Biochemical Composition						
Collagen (mg/g bone)	212	216	199*	215	218	225
Hydroxyproline (HYD)[1]	90	92	97	93	97	92
Proline (PRO)[1]	105	108	112	105	113	107
PRO:HYD ratio[1]	1.16	1.18	1.16	1.14	1.16	1.16
HYD (mg/g bone)	16	16	16	16	16	17
PRO (mg/g bone)	22	23	23	23	23	24
PRO:HYD ratio (bone)	1.42	1.42	1.43	1.44	1.41	1.40
Calcium (mg/g bone)	215	220	221	221	221	219
Phosphorus (mg/g bone)	129	133	134	133	131	130
Ca + P/Collagen	1.63	1.64	1.79	1.67	1.62	1.56
Density (mg/cm^3)	206	212	212	214	211	198
Mechanical Properties						
Rupture ($g\text{-force}/mm^2$)	439	452	431	423	419	389
Elastic Limit ($g\text{-force}/mm^2$)	399	410	391	383	390	355
% Strain	5.8	5.7	6.1	5.9	5.5	5.5
Modulus of Elasticity ($\frac{kg\text{-force}}{mm^2}$)	9.89	10.00	9.01	9.46	9.42	8.64*
Toughness ($g\text{-}mm/m^3$)	15.1	15.4	15.4	14.9	13.8	12.5**
Fish Size						
Length (mm)	63	66*	65	63	69**	68**
Weight (g)	2.04	2.45	2.23	2.07	2.80**	2.56**
Number of Fish	8	8	8	8	8	8

[1] Measured as mg/g collagen; ratio of PRO:HYD in collagen.

Fish for all three chronic toxicity tests were taken from the same lot of striped bass so bone development analyses from the three tests actually tracked the growth of a single cohort. Comparison of bone development characteristics of control fish over the three tests is presented in Table 23. Significant biochemical changes with increasing fish size/age occurred for proline, hydroxyproline, and their ratio in collagen. Phosphorus concentration and mineral (Ca + P):collagen ratio decreased significantly in successively older fish. Bone density, rupture, elastic limit and modulus of elasticity all increased in older fish while percent strain and bone toughness decreased (Table 23).

RNA and DNA analyses of striped bass collected during each chronic toxicity test indicated no relationship between RNA:DNA ratio and any copper or cadmium concentration. During Chronic Toxicity Test #1, RNA:DNA ratios declined from a range of 1.9-2.3 for 25-day-old fish to a range of 1.1-1.4 for 58 day-old-fish, over all copper concentrations and the controls. The range of ratios in Toxicity Test #3 was 1.9-2.7 for 75-day-old juveniles, 1.6-2.0 for 90-day-old fish, and 1.3-1.9 in juveniles at 135 days post-hatch. None of the variation in ratios was significant between copper concentrations and the controls even though the range of ratios again decreased with increasing age of test fish. Nucleic acid ratios in Chronic Toxicity Test #2 varied from 1.2 to 2.2 with no apparent trends related to cadmium concentration or age of striped bass.

Table 23. Means for biochemical composition, density and mechanical properties of vertebral bone from control striped bass in chronic toxicity tests with copper or cadmium. Values with superscripts in common across rows are not significantly different (P>0.05).

	Chronic Toxicity Test Number		
	1	2	3
Age of Fish Sampled (Days)	48	105	135
Biochemical Composition			
Collagen (mg/g bone)	184[a]	212[a]	212[a]
HYD[1] (mg/g collagen)	65[a]	90[b]	57[c]
PRO[2] (mg/g collagen)	84[a]	105[b]	82[a]
PRO:HYD (collagen)	1.31[a]	1.16[b]	1.43[b]
HYD (mg/g bone)	16[a]	16[a]	15[a]
PRO (mg/g bone)	24[a]	22[a]	23[a]
PRO:HYD (bone)	1.48[a]	1.42[a]	1.51[a]
Calcium (mg/g bone)	221[a]	215[a]	198[a]
Phosphorus (mg/g bone)	153[a]	129[b]	115[a]
Ca + P/Collagen	1.90[a]	1.63[b]	1.49[c]
Density (mg/cm^3)	*	206[a]	243[b]
Mechanical Properties			
Rupture ($g\text{-force}/mm^2$)	*	439[a]	454[a]
Elastic Limit ($g\text{-force}/mm^2$)	*	399[a]	422[a]
% Strain	*	5.8[a]	4.8[b]
Modulus of Elasticity ($\frac{Kg\text{-force}}{mm^2}$)	*	9.89[a]	11.97[b]
Toughness ($g\text{-}mm/m^3$)	*	15.1[a]	12.8[b]
Fish Size			
Length (mm)	41[a]	63[b]	84[c]
Weight (g)	0.67[a]	2.04[b]	6.26[c]
Number of Fish	8	8	8

[1] HYD = hydroxyproline
[2] PRO = proline
*Small size of fish precluded determination.

Discussion

Acute toxicity of copper and cadmium to larval striped bass in this investigation was similar to previously reported acute tests of this species in a variety of background water qualities and with fish of different ages and sizes. Published estimates of 96-hour LC50's for copper tested with larval striped bass range from 50 µg/l for 7-day-old fish in reconstituted water (Hughes 1969, 1973) to 270 µg/l for 35-day-old fish in hard well water (Mehrle and Ludke 1983). The range of 96-hour LC50's for copper in this study was 34 µg/l with 3-day-old fish in soft, dechlorinated tapwater to 463 µg/l with 9-day-old larvae at 5%. salinity. This variation in copper toxicity to larval striped bass in waters differing in pH, hardness, alkalinity, and other factors is consistent with the well known effects of these water quality parameters on the toxicity of copper to fish (e.g. Chakoumakos et al. 1979; EIFAC 1976; Howarth and Sprague 1978; Miller and Mackay 1980). Increased hardness and alkalinity tend to reduce the acute toxicity of copper, although the interaction of these factors with pH and metal speciation can produce complex changes in toxicity (Chakoumakos et al. 1979; Howarth and Sprague 1978). Nevertheless, the range of acute toxicity (96-hour LC50's) between hard and soft water reported here for larval striped bass is very close to the range of 20-520 µg/l total dissolved copper noted by Howarth and Sprague (1978) for young rainbow trout (Salmo gairdneri) in soft and hard water. Mehrle and Ludke (1983) found that copper was more than twice as toxic to striped bass fry compared to rainbow trout fry in the same reconstituted water.

Organic complexing agents are water quality constituents which also affect copper toxicity to fish (Buckley 1983; Pagenkopf 1983; Pagenkopf et al. 1974) and other aquatic biota (Harrison 1985; Sunda and Lewis 1978). However, organic chelators were unlikely factors in the present striped bass study because all of the test water was filtered through activated

charcoal which removes a large fraction of dissolved organics involved in copper complexation (Buckley 1983).

Fish age/size was an important influence on copper lethality to striped bass larvae. Acute toxicity increased with increasing age for larvae 11-20 days old, in contrast with the inverse relationship reported between copper toxicity and the size/age of rainbow trout fry (Howarth and Sprague 1978). Greater copper toxicity to older striped bass larvae is likely related to developmental changes which increase sensitivity such as development of gill surface area or ion exchange capacity leading to enhanced metal toxicity (Pagenkopf 1983). Juvenile striped bass are more tolerant than larvae to copper exposure, with 96-hour LC50 estimates of 150-620 µg/l in freshwater (Hughes 1971; Wellborn 1969) and 4,300 µg/l in estuarine water (Rehwoldt et al. 1971). Increased tolerance for toxicants among larger fish is consistent with the literature on most contaminants, with some exceptions (Howarth and Sprague 1978).

Cadmium acute toxicity to striped bass larvae in this study spanned a wider range than the reported 96-hour LC50 range of <1.0 µg/l (Hughes 1973) in reconstituted freshwater to 75 µg/l in 1%. salinity as Instant Ocean® (Mehrle and Ludke 1983). However, the present investigation included as many acute tests with larvae and cadmium as all of those previously reported combined. Cadmium toxicity to striped bass larvae reported here is consistent with the results of Wright et al. (1985) that increased calcium concentration reduced cadmium toxicity to 7-day-old striped bass larvae. The data in Table 7 indicate a large decrease in cadmium toxicity to 3-day-old larvae between freshwater (similar to the low calcium water of Wright et al.) and 1.0%. salinity as Instant Ocean® which contains approximately 75 mg/l calcium (Mehrle and Ludke 1983) - similar to the high calcium water of Wright et al. (1985). The data in Table 7 also support these authors' indication that cadmium toxicity to larval striped bass is variable and changes with stage of development. Cadmium toxicity to post yolk sac fish in this report

significantly increased in tests with larvae older than 11 days post-hatch (Tables 4 and 7) in a similar pattern to increased copper toxicity to older larvae (Tables 3 and 6). Increasing metal toxicity with increasing age of young striped bass after yolk sac absorption may explain the greater sensitivity to copper and cadmium among 35-80 day-old fish (Mehrle and Ludke 1983) in comparison with the 3-20 day-old fish reported here. Chapman (1978) noted that cadmium (and copper) toxicity was greater for steelhead (Salmo gairdneri) and chinook salmon (Oncorhynchus tshawytscha) swim-up alevins and parr than for newly hatched alevins.

The age, size, and apparent biological competency of striped bass juveniles made a large difference in the sensitivity of these fish to acute metal exposure. Substantially reduced cadmium toxicity to older fish in this report and that noted by Wright et al. (1985) for juvenile striped bass agree with the reduction in toxicant sensitivity among larger organisms in general (Anderson and Weber 1975).

Hyperactivity preceding tetany noted among some juvenile striped bass exposed to cadmium closely approximated the general symptoms of cadmium poisoning in other fishes (Benoit et al. 1976; Peterson et al. 1983). The proposed mechanism producing such symptoms is related to antagonistic calcium-cadmium interactions, which also explain the protective effect of calcium on cadmium acute toxicity (Carroll et al. 1979).

The chronic toxicity test of copper with striped bass fry was a more complex experiment than originally designed because of the presence of larvae lacking an inflated swimbladder. Larval striped bass with this anomaly have been described (Bulak and Heidinger 1980; Cornacchia 1981; Doroshev 1970; Doroshev and Cornacchia 1979) and are mentioned as a consistent problem among intensively cultured fish (Carlberg et al. 1984; Lewis and Heidinger 1981). However, this is the first report on survival and growth of such fish in toxicity tests. Uninflated larvae were much less sensitive to 50 µg/l copper than normal, inflated

fish after 30 days' exposure, probably due to their slower growth and aberrant development (Doroshev 1970). Increased mortality of uninflated fish between days 30 and 48 of this test at all copper concentrations and the controls duplicates Doroshev's (1970) observations on the timing of mortality among uninflated fry transported long distances by air as prolarve.

A no observable effect concentration or MATC for copper after 30 days' exposure of striped bass larvae was 25 µg/l, based on combined survival of fish with and without an inflated swimbladder. This concentration is similar to the range of 31.7-43.5 µg/l which caused significant reduction in larval standing crops of seven freshwater fish species treated with copper for 60 days (McKim et al. 1978). The MATC for striped bass juveniles tested with copper was 50 µg/l. As with acute toxicity, sensitivity to chronic copper exposure was highest for striped bass larvae and early juvenile stages. Results with striped bass sensitivity to other toxicants have also demonstrated this phenomenon (Burton et al. 1979; Hall et al. 1984).

Growth was not an indicator of sublethal copper effects on striped bass in this investigation. Acclimation to sublethal copper concentrations during the first weeks of each test is the most plausible explanation for this result. Dixon and Sprague (1981a) found that prior acclimation to sublethal copper levels by rainbow trout significantly affected growth and subsequent lethal tolerance to copper among acclimated fish. Woltering (1984) noted that the growth response of fish fry as a chronic toxicity endpoint, was reduced at the lowest effect concentration in only 36% of the partial chronic early life stage toxicity tests he reviewed. Woltering concluded that fish growth response to toxicants could be deleted from early life stage toxicity tests without significant impacts on chemical hazard assessments.

Cadmium concentrations tested in Chronic Toxicity Test #2 had no impact on survival or growth of striped bass because the test concentrations were based on cadmium acute toxicity to 20 day-old larvae which were much more sensitive to cadmium than the

older (45-day post-hatch) fish used in the test. From this experiment, it appears that juvenile striped bass reach a point in development at and after which their tolerance to cadmium (and copper) exposure dramatically increases. In fact, this experiment demonstrated that for juvenile fish, cadmium levels of 10 µg/l may even enhance growth (Table 16), although such results are likely only with relatively high background calcium concentrations (Wright et al. 1985). Significant growth reduction in Atlantic salmon alevins occurred in freshwater tests at cadmium concentrations as low as 0.47 µg/l (Rombough and Garside 1982).

Total copper and cadmium concentrations measured in test waters during this study can be considered as directly proportional to concentrations of the divalent ion of each metal. McCrady and Chapman (1979) demonstrated essentially linear relationships between total copper and cupric ion concentrations in reconstituted and well waters containing few if any organic complexing agents. Concentrations of other major ions present in test water were not measured directly during this investigation but were on the order of those presented by Spotte (1970) for Instant Ocean®, in all tests involving brackish salinities. This ionic composition approximates that of natural sea water.

Bioaccumulation of copper by striped bass was consistent with that reported for other fishes (Benoit 1975; Dixon and Sprague 1981b; Seim et al. 1984) in being directly proportional to chronic exposure concentration. The body burdens of copper reported in striped bass from this research can be considered equilibrium values in relation to copper levels in water because duration of exposure equaled (Chronic Toxicity Test #1) or exceeded (Chronic Test #2) that deemed necessary to reach steady-state conditions (Hamelink 1977; Veith et al. 1979). Tissue copper concentrations from young striped bass in this study are very similar to those for juvenile rainbow trout in relation to copper dosage (Dixon and Sprague 1981b). Another major similarity between these data is the dramatic increase in copper

body burden in trout and striped bass at the lowest exposure concentration which significantly reduced survival. Comparable results have been obtained by Benoit (1975) with bluegills (Lepomis macrochirus) and by McKim and Benoit (1974) with brook trout (Salvelinus fontinalis). Copper as a physiologically regulated and essential metal (Giesy and Wiener 1977; Vinikour et al. 1980) in fish could be expected to increase in individuals exposed to concentrations that lethally overcome metabolic regulation. Heavy metal residues documented in striped bass from wild populations, including the Sacramento-San Joaquin stock, have been summarized by Rogers et al. (1980).

Cadmium accumulation by young striped bass in direct proportion to cadmium exposure in this study coincides with bioaccumulation results from chronic tests with young salmonids (Peterson et al. 1983; Rombough and Garside 1982). Data on short-term (120-hour) bioaccumulation of cadmium by larval striped bass (Wright et al. 1985) are difficult to compare with the present test because brief exposure periods are not considered sufficient to establish steady-state conditions for bioaccumulation of most contaminants (Hamelink 1977) nor to reach maximum tissue concentrations of cadmium in fish (Cearley and Coleman 1974). Cadmium accumulation by juvenile striped bass may be higher than that reported here because none of the cadmium concentrations in this study affected growth or survival of test fish.

Bioconcentration factors for copper and cadmium in juvenile striped bass were relatively low in comparison with those reviewed by Veith et al. (1979) for a variety of organic compounds tested with various freshwater fishes. Cadmium bioconcentration factors were close to those for subadult striped bass held for 28 days in Central Valley agricultural drainage water and Sacramento-San Joaquin water (MBL 1983).

Significant reduction in the calcium content of vertebrae from striped bass fry after chronic exposure to copper coincides with significantly lower calcium levels in young-of-the-year

striped bass from the Hudson River-the most polluted river from which juvenile fish were sampled for comparison of bone development (Buckley et al. 1985). Depressed calcium levels also occurred in rainbow trout fry exposed to mixtures of arsenic and selenium (Hunn et al. In Review). However, changes in calcium content of copper-exposed striped bass larvae were not repeated in tests with older juveniles. In addition, mechanical properties of juvenile vertebrae were enhanced at sublethal copper concentrations, in contrast to the reduced bone strength ofjuvenile striped bass from the Hudson River (Mehrle et al. 1982). Overall, data on bone development of young striped bass indicated that vertebral biochemistry and mechanics are not as sensitive an indicator as survival in denoting copper concentrations that may adversely affect these fish.

Modulus of elasticity was the only bone development characteristic significantly reduced in young striped bass treated with cadmium. This mechanical property was also comparatively low in striped bass juveniles sampled from the Hudson River (Buckley et al. 1985; Mehrle et al. 1982), and was the only measure of bone strength significantly reduced in juvenile fish tested with copper.

Biotic and abiotic factors in Bay-Delta striped bass habitats may serve to enhance or ameliorate adverse effects of copper and cadmium demonstrated here. However, this study has demonstrated that the striped bass life history stages most sensitive to copper and cadmium, overlap the developmental period during which relative year-class strength is determined (Chadwick et al. 1977; Stevens 1977). Better information is needed on the interactions of young striped bass with major nursery habitat components in the Bay-Delta, in order to accurately assess the effects of contaminants on early survival and successful recruitment.

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