ACUTE TOXICITY OF CHLOROFORM TO BLUEGILL (*Lepomis macrochirus*), RAINBOW TROUT (*Salmo gairdneri*), AND PINK SHRIMP (*Penaeus duorarum*).

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INTRODUCTION

The current concern regarding the protection of aquatic life in surface waters has prompted the evaluation of the effects of exposure to chemicals on aquatic organisms. The primary objective of these studies was to provide the Environmental Protection Agency with information to evaluate the relative susceptibility of aquatic organisms to acute exposure to chloroform. The acute toxicity of chloroform to bluegill and rainbow trout in both a soft and a hard water, and pink shrimp in sea water was estimated during static bioassays.

The bioassays with fishes were conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts. The shrimp bioassay was conducted at the Marine Research Laboratory of E G & G, Bionomics, Pensacola, Florida.

MATERIALS AND METHODS

The methodology for acute toxicity testing of fishes and shrimp closely followed the recommended bioassay procedures as described in Standard Methods (APHA, 1971) except for certain conditions described below.

The chemical evaluated in these bioassays was reagent grade chloroform ($CHCl_3$), a clear liquid manufactured by Mallinckrodt Chemical Company (lot #WBRC) tested as 100% active ingredient. Results for all tests were expressed as the median lethal concentration (LC50), the nominal concentration of the test compound in water causing 50 percent mortality of test animals. The LC50 value and its 95% confidence interval were calculated by converting the test concentrations and the corresponding observed percent response to logs and probits, respectively. These values were then utilized in a least squares regression analysis, and the LC50 value and its confidence interval were estimated from the calculated regression equation.

The animals used in these tests were bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri) and pink shrimp (Penaeus duorarum). The bluegill were acquired from

a commercial fish hatchery in Nebraska, and had a mean wet weight of 1.1 g and a mean standard length of 37 mm. The rainbow trout were obtained from a commercial fish farmer in Washington, and had a mean wet weight of 1.0 g and a mean standard length of 32 mm. The shrimp were collected by laboratory personnel from Big Lagoon in Pensacola, Florida and had rostrum-telson lengths of 35-50 mm.

The bluegill and rainbow trout were held in 1700-l concrete raceways which are coated with an epoxy resin paint to prevent leaching of materials into the water. Flow of well water (having a temperature of 21 $\pm$ 1.0°C for the bluegill, and 12 $\pm$ 1.0°C for the rainbow trout) into these raceways was at a minimum flow of 4 l/minute, providing an adequate rate of turnover for holding these species. This water had a hardness of 35 mg/l as $CaCO_3$, a pH of 7.1 and a dissolved oxygen concentration of at least 6.0 mg/l (60% of saturation). These species were maintained in the laboratory hatchery facilities for at least 30 days prior to testing. During the 30 day period, mortality was <2%; no mortality was observed during the 48 hours immediately prior to testing, and these fish were judged to be in ex-

cellent condition. The shrimp were held in 1100-l fiberglass tanks in constantly flowing filtered (10 micrometers) natural sea water. The salinity of this water was 25 part per thousand (o/oo) and the temperature was 20 $\pm$ 1.0°C.

The static bioassays were conducted in 19.6-l wide-mouth soft-glass bottled containing 15 liters of test solution. Exposure mixtures for the bluegill bioassays were maintained in water baths at 21 $\pm$ 1.0°C by immersion coil heaters and mercury column thermoregulators. Test solutions for the rainbow trout and shrimp were maintained in water baths at 12 $\pm$ 1.0°C and 20 $\pm$ 1.0°C, respectively by use of commercial refrigeration units. Each species was from the same year class, and the standard length of the longest fish or shrimp was no more than two times that of the shortest fish or shrimp. The bluegill and rainbow trout were acclimated to test conditions of temperature and water quality over a 96-hour period prior to testing. These species were not fed during the 48 hours immediately prior to testing or during the tests. The shrimp were acclimated to test conditions of water quality and temperature for at least seven days prior to testing. Water in the test vessels was not aerated. In all bioassays, the test compound was pipeted directly into each jar. Animals were introduced into the test vessel within 30 minutes after the compound

was added. Ten bluegill or rainbow trout were randomly assigned to each test vessel. Ten shrimp (2 replicates, 5 animals/vessel) were exposed to each concentration.

The dilution water used in the fish bioassays was the same as previously described for holding these fish. The hard water for these bioassays was prepared by adding 192 mg of $NaHCO_3$, 120 mg of $CaSO_4$, 120 mg of $MgSO_4$, and 8 mg of KCl per liter of deionized water. The resulting water had a pH of 7.6 and a total hardness of 200 mg/l as $CaCO_3$. The dilution water for the shrimp bioassay consisted of filtered (10 micrometers) natural sea water with a salinity of 25 o/oo and a pH of 8.0 $\pm$ 0.5. Concentrations of dissolved oxygen were measured with a combination temperature-oxygen probe and meter in selected concentrations at 0, 24, 48 and 96 hours of exposure.

Two series of concentrations were established within a bioassay, a series of range-finding (preliminary) concentrations and a series of definitive concentrations. The preliminary test was conducted to determine the approximate range of concentrations for evaluating the dose-response relationship. The definitive test, consisting of at least five concentrations evaluated the dose-response relationship

to a degree allowing the LC50 to be calculated from the data with optimum accuracy. A control, which consisted of the same dilution water, conditions, procedures, and organisms, was maintained for each species tested.

RESULTS AND DISCUSSION

The estimated LC50 values and 95% confidence intervals are presented in Table 1 along with the highest nominal concentration at which there were no discernible effects on test animals due to exposure to chloroform. A summary of observed mortality for each individual test concentration at 24, 48 and 96 hours of exposure to chloroform is also presented (Table 2). The mortality syndrome among fish from those concentrations where mortality was observed was similar. Fish generally became dark and lethargic, lost equilibrium, and expired. The shrimp exposed to the test concentrations of 56 and 100 mg/l became lethargic during the first 24 hours of exposure and were often observed lying on their sides during the test. However, after 96 hours of exposure, all nine remaining shrimp in the 56 mg/l concentration were upright and appeared in good condition; in the 100 mg/l concentration, five of the seven remaining shrimp were on their

sides and two were upright. These effects could have been the result of volatilization of chloroform, and subsequent reduced concentrations in test water, over the 96-hour period. The concentrations of dissolved oxygen, measured at 0, 24, 48 and 96 hours of exposure, are presented in Table 3. Final pH was 7.0 $\pm$ 0.5 for all test concentrations and controls where bluegill and rainbow trout were exposed in soft water. Comparable pH's for the test concentrations where bluegill and rainbow trout were exposed in hard water were 7.5 $\pm$ 0.5. Final pH was 8.0 $\pm$ 0.5 for all test concentrations and controls for the shrimp bioassay.

The LC50 values for those bioassays exposing bluegill in both soft and hard water were essentially the same after 96 hours of exposure. The 96-hour value for rainbow trout in soft water was ca 1.5X that for rainbow trout in hard water. The LC50's for shrimp at 48 and 96 hours of exposure were between the LC50 values determined for bluegill and rainbow trout.

LITERATURE CITED

A.P.H.A. 1971. Standard Methods for the Examination of Water and Wastewater. 13th Edition, 874 pp.

Table 1 -- Acute toxicity of chloroform to bluegill[a] (Lepomis macrochirus), rainbow trout[b] (Salmo gairdneri), and pink shrimp[c] (Penaeus duorarum). These data are based on the results of bioassays conducted at the Aquatic Toxicology Laboratory and the Marine Research Laboratory of E G & G, Bionomics, Wareham, Massachusetts and Pensacola, Florida.

Species/ diluent	LC50 (mg active ingredient/l) 24 hour	48 hour	96 hour	No discernible effect level at 96 hours (mg/l)
bluegill/ soft water	185 (155-221)[d]	123 (107-143)	115 (96-138)	100
bluegill/ hard water	119 (96-148)	100 (72-140)	100 (72-140)	75
rainbow trout/ soft water	67.5 (55.2-82.5)	67.5 (55.2-82.5)	66.8 (54.8-81.4)	42.0
rainbow trout/ hard water	55.5 (40.7-75.7)	43.8 (36.1-53.2)	43.8 (36.1-53.2)	24.0
pink shrimp	134 (96.3-187)	81.5 (62.8-106)	81.5 (62.8-106)	32.0

a Bioassays conducted at 21 ± 1.0°C, mean wet weight of bluegill 1.1 g.

b Bioassays conducted at 12 ± 1.0°C, mean wet weight of rainbow trout 1.0 g.

c Bioassays conducted at 20 ± 1.0°C, rostrum-telson lengths of pink shrimp 35-50 mm.

d 95% confidence interval.

Table 2 -- Concentrations tested and corresponding observed percentage mortalities at 24, 48 and 96 hours for bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), and pink shrimp (Penaeus duorarum) exposed to chloroform.

Species/ diluent	Nominal concentration (mg/l)	% mortality observed 24 hour	48 hour	96 hour
bluegill/ soft water	320	100	100	100
	240	60	100	100
	180	80	100	100
	140	10	80	100
	120	0	90	90
	100	0	0	0
	control	0	0	0
bluegill/ hard water	320	100	100	100
	240	100	100	100
	180	100	100	100
	140	30	100	100
	100	30	40	40
	75	0	0	0
	control	0	0	0
rainbow trout/ soft water	100	100	100	100
	75	30	30	40
	56	10	10	10
	42	0	0	0
	32	0	0	0
	control	0	0	0

Table 2 -- Continued.

Species/ diluent	Nominal concentration (mg/l)	% mortality observed 24 hour	48 hour	96 hour
rainbow trout/ hard water	100	100	100	100
	75	100	100	100
	56	40	70	70
	42	0	20	20
	32	0	10	10
	24	0	0	0
	control	0	0	0
pink shrimp	320	100	100	100
	180	90	100	100
	100	10	30	30
	56	10	10	10
	32	0	0	0
	control	0	0	0

Table 3 -- Measured concentrations of dissolved oxygen during 96-hour exposures of bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), and pink shrimp (Penaeus duorarum) to chloroform.

Species/ diluent	Nominal concentration (mg/l)	Dissolved oxygen (mg/l and % of saturation)			
		0 hour	24 hour	48 hour	96 hour
bluegill/ soft water	320	7.3(81)	-[a]	-	-
	240	7.7(87)	6.8(75)	5.2(57)	4.7(52)
	100	7.4(82)	6.6(73)	4.8(53)	4.2(46)
	control	7.5(85)	5.8(64)	4.7(52)	4.0(44)
bluegill/ hard water	180	8.0(90)	-	-	-
	100	8.1(91)	5.8(64)	4.2(46)	3.9(43)
	75	7.8(88)	5.2(56)	4.6(51)	4.0(44)
	control	7.7(87)	6.3(70)	5.8(64)	4.1(45)
rainbow trout/ soft water	100	9.2(85)	-	-	-
	75	9.3(86)	7.7(70)	7.2(66)	6.9(63)
	42	9.0(82)	7.9(72)	7.5(69)	7.1(65)
	control	9.0(82)	7.8(71)	6.8(62)	6.5(60)
rainbow trout/ hard water	75	9.0(82)	-	-	-
	56	9.8(90)	9.8(90)	7.4(67)	6.8(62)
	32	9.1(84)	8.4(78)	6.9(63)	6.1(56)
	control	9.7(89)	9.7(89)	6.6(60)	6.1(56)

Table 3 -- Continued.

Species/ diluent	Nominal concentration (mg/l)	Dissolved oxygen (mg/l and % of saturation) 0 hour	24 hour	48 hour	96 hour
pink shrimp/ sea water	180	6.8(89)	5.6(74)	-[a]	-
	100	6.7(87)	6.3(83)	5.8(76)	3.0(39)
	32	6.8(89)	6.2(82)	5.7(75)	4.0(53)
	control	6.8(89)	6.8(89)	6.0(79)	3.5(46)

[a] Dissolved oxygen not measured due to 100% mortality.