(31)

EFFECTS OF AROCLOR® 1016 ON EMBRYO, FRY, JUVENILE AND ADULT SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS)[1]

David J. Hansen, Steven C. Schimmel and Jerrold Forester

U. S. Environmental Protection Agency

Gulf Breeze Environmental Research Laboratory

Sabine Island, Gulf Breeze, Florida 32561

(Associate Laboratory of the

National Environmental Research Center,

Corvallis, Oregon)

March 6, 1974

®Registered trademark, Monsanto Company, St. Louis, Missouri.

Mention of commercial products or trade names does not constitute endorsement by the Environmental Protection Agency.

1/Contribution No. 206 from the Gulf Breeze Environmental Research Laboratory, U. S. Environmental Protection Agency, Gulf Breeze, Florida 32561

DSW 028785

STLCOPCB4012747

EFFECTS OF AROCLOR® 1016 ON EMBRYO, FRY, JUVENILE AND ADULT SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS)

David J. Hansen, Steven C. Schimmel and Jerrold Forester

ABSTRACT

We investigated the toxicity of Aroclor 1016 to, and its uptake by, fry, juvenile and adult sheepshead minnows (Cyprinodon variegatus) in intermittent-flow bioassays lasting 28 days. Fry, juvenile and adult fish apparently were not affected by 0.1, 0.32, 1.0, 3.2 or 10 µg/l, but died in 32 and 100 µg/l (ppb) of this polychlorinated biphenyl. Sheepshead minnows accumulated the chemical in proportion to its concentration in the test water. Fry contained 2,500 to 8,100 X the nominal concentration in the test water, adults 4,700 to 14,000 X and juveniles 10,000 to 34,000 X. As much as 77 µg/g (ppm) of Aroclor 1016 in eggs from exposed adults apparently did not affect survival of embryos and fry.

DSW 028786

STLCOPCB4012748

INTRODUCTION

Polychlorinated biphenyls, PCB's, occur in estuarine environments in the United States (Butler, 1973; Nimmo and Bahner, 1974a). One PCB, Aroclor 1254, is acutely toxic (48 or 96 hours) to certain estuarine animals (Duke et al., 1970) and in exposures lasting 14 or more days it is toxic to some estuarine arthropods (Nimmo et al., 1971; Nimmo et al., 1974b), mollusks (Lowe et al., 1972) and fishes (Hansen et al., 1971; Schimmel et al., 1974). It is over 30 times more toxic to fry of sheepshead minnows (Cyprinodon variegatus) than to juveniles or adults (Schimmel et al., 1974). Fry from sheepshead minnow eggs containing Aroclor 1254 die within the first week following hatching (Hansen et al., 1974a).

A new PCB, Aroclor 1016, is now being manufactured to replace some of the PCB's that are no longer produced. Aroclor 1016 has not been found in oysters or fishes from estuaries in the United States sampled by the Environmental Protection Agency's National Estuarine Monitoring Program (P. A. Butler, personal communication[2/]). It is however, as acutely toxic to some juvenile and adult estuarine animals as are other PCB's and its delayed toxicity to pinfish (Lagodon rhomboides) in exposures lasting 14 or more days is similar to that found with another PCB (Hansen et al., 1974b).

2/ P.A. Butler, Ecological Monitoring Branch, Technical Services Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Gulf Breeze, Florida 32561.

STLCOPCB4012749

This experiment was conducted to determine the toxicity of Aroclor 1016 in water to embryo, fry, juvenile and adult sheepshead minnows and its endogenous toxicity to embryos and fry reared from eggs from exposed adults. Also, chemical analyses were made to determine if this PCB accumulates in eggs, fry, juvenile and adult fish.

MATERIALS AND METHODS

Test Fish

Adult and juvenile fish were collected near the Gulf Breeze Laboratory and acclimated in 30 C seawater for at least 14 days before exposure. Fish were not used if mortality exceeded 1% or abnormal behavior was observed in the 48 hours immediately preceding a test. Adult fish averaged 41mm standard length (range 31-51mm) and juveniles averaged 20mm standard length (range 16-27mm). Fish were fed PCB-free dry commercial fish food daily and PCB-free frozen adult brine shrimp twice a week during acclimation and testing.

Eggs were obtained from acclimated adult females whose egg production was enhanced by hormonal injection using the techniques of Schimmel et al. (1974). Eggs, stripped manually from six or more females, were placed in 20-40ml of filtered 30 C seawater and fertilized with excised macerated testes from six or more males. Success of fertilization was determined by microscopic examination for cleavage 1.5 hours after mixing with macerated testes. Fry from the eggs were fed daily on PCB-free brine shrimp nauplii.

Aroclor 1016 exposure

Adult, juvenile, embryo and fry sheepshead minnows were exposed

STLCOPCB4012750

simultaneously to Aroclor 1016 in two intermittent-flow bioassays, each lasting four weeks. In the first experiment, we exposed 25 adults (13 females and 12 males), 25 juveniles and 100 eggs in aquaria receiving 0, 0.1, 0.32, 1.0, 3.2, or 10 µg/ℓ of Aroclor 1016. In the second experiment exposure concentrations were 0, 1.0, 3.2, 10, 32, and 100 µg/ℓ. The dosing apparatus (Schimmel et al., 1974) was a modification of that of Brungs and Mount (1970). Each of approximately 150 daily cycles siphoned 1.5% of filtered 30 C seawater, 11 µg of carrier (polyethylene glycol 200) and appropriate amounts of PCB to each 80ℓ aquarium. Water and carrier without PCB were delivered to the control aquaria. Salinity of the water averaged 22.7‰ (12.0‰ to 32.0‰). Aquaria were checked daily to determine survival of embryo, fry, juvenile and adult fish.

Aroclor 1016 in eggs

The effect of Aroclor 1016 in eggs was determined during the second bioassay by enhancing egg production with hormonal injections of exposed adults, fertilizing the eggs artificially, then monitoring their development in flowing PCB-free seawater. Female sheepshead minnows were injected intraperitioneally with 50 I.U. human chorionic gonadotropic hormone on exposure days 25 and 27. On day 28 or 29, eggs were stripped manually from five females from each concentration and those from each female placed in individual beakers containing 20-40 ml of filtered 30 C seawater. Eggs from each female were fertilized with excised macerated testes from a male from the same concentration. Twenty-five eggs from each fish were placed in a 10 cm Petri dish to which a 9 cm-high collar of 500µ nylon

STLCOPCB4012751

mesh was attached. Dishes were submerged 7 cm in the 80 % control aquarium which received approximately 225 % of filtered 30 C PCB-free seawater per day; average salinity was 24.0% (19.5% to 28.%). Success of fertilization was determined by microscopic examination for cleavage 1.5 hours after mixing with macerated testes. Thereafter, dishes were examined daily for 21 days to determine survival of embryos and fry. Fry were fed PCB-free live brine shrimp nauplii daily.

Chemical analyses

Concentrations of Aroclor 1016 in water and fish were determined by electron-capture gas chromatography. Unfiltered water samples from each concentration were obtained at least three times during each four-week exposure and analyzed. Concentrations of Aroclor were determined in fry, juvenile and adult fish surviving both bioassays and in eggs from adult fish from the second bioassay. All fish samples consisted of 14 or more individuals. Procedures used for water and fish samples are described by Hansen et al. (1974b). Aroclor 1016 was quantitated by comparing the total height of all peaks in the sample with the total height of all peaks in a standard of known concentration. Recoveries were greater than 80%; data were not adjusted for recovery. All tissue residues were determined on a wet-weight basis.

Statistical analyses

Concentrations of Aroclor 1016 lethal to 50% of the fish (95% confidence limits) were estimated using the method of Litchfield and Wilcoxon (1949). The χ^2 test for independent samples was used to compare the survival of control and PCB-exposed fish. Differences were considered significant at $\alpha = 0.05$.

STLCOPCB4012752

RESULTS AND DISCUSSION

Sheepshead minnow adults, juveniles, embryos and fry exposed to Aroclor 1016 for 28 days apparently were not affected by 0.1, 0.32, 1.0, 3.2 or 10 µg/ℓ, but died in 32 and 100 µg/ℓ, nominal concentrations, of this PCB (Table 1). Response to poisoning included darkened body coloration, uncoordinated swimming, cessation of feeding and lesions on the body of many juveniles and adults. Juvenile pinfish, another estuarine fish, also died in 32 µg/ℓ of Aroclor 1016, but survived lower concentrations (Hansen et al., 1974b); responses exhibited by both fishes were similar. Although fin was not observed in Aroclor 1016-exposed fish (present study; Hansen et al., 1974b) it was common in Aroclor 1254-exposed sheepshead minnows (Schimmel et al., 1974), spot (Leiostomus xanthurus) and pinfish (Hansen et al., 1971).

Sensitivities of embryos and fry, juvenile and adult sheepshead minnows to Aroclor 1016 were similar (Tables 1 and 2). Aroclor 1016 did not alter the survival of embryos to hatching or the hatching time. Eggs began to hatch on the fourth day of exposure, one-half hatched by day five and all hatched by day nine. Fry, juveniles and adults in 32 and 100 µg/ℓ began to die during the second week of exposure and over half died before the end of the second week. All fish in 100 µg/ℓ were dead after 3 weeks, but some fish of each life stage survived the four-week exposure to 32 µg/ℓ. Statistical analyses of weekly data show the similiarity of effects of Aroclor 1016 on each life stage (Table 2). In similar experiments, Schimmel et al. (1974) reported that Aroclor 1254 affected each of these life stages differently: Sheepshead minnow fry were much more sensitive than other stages.

STLCOPCB4012753

During three-weeks exposure, 0.32 µg/ℓ of Aroclor 1254 was lethal to fry, 10 µg/ℓ was lethal to juveniles and adults survived 10 µg/ℓ.

Sheepshead minnows exposed for 28 days to concentrations of Aroclor 1016 ranging from 0.1 and 100 µg/ℓ accumulated the chemical in proportion to the concentration in the test water (Table 3). In general, juvenile fish contained the greatest concentrations of the PCB, followed by adults and fry (fry were exposed 5 days as embryos followed by 28 days as fry). Concentration factors (concentration in whole-fish divided by nominal concentration in the test water) ranged from 2,500 to 8,100 for fry, 4,700 to 14,000 for adults and 10,000 to 34,000 for juveniles. Concentration factors in adult sheepshead minnows exposed identically to Aroclor 1254 were greater (15,000 to 30,000) (Hansen et al., 1974a). By comparison, concentration factors in fry and adult sheepshead minnows exposed for 3 weeks to 0.1, 0.32, 1.0, 3.2 or 10 µg/ℓ of Aroclor 1254 were similar, ranging from 11,000 to 32,000 (Schimmel et al., 1974).

Eggs from sheepshead minnows exposed to 1.0, 3.2 or 10 µg/ℓ of Aroclor 1016 for 4 weeks contained up to 77 µg/g of the PCB (Table 4). Fertilization success, survival of embryos to hatching and survival of fry for two weeks after hatching did not appear to be altered by this or lesser concentrations. Sheepshead minnow eggs that contained Aroclor 1254 in concentrations of 7 µg/g or more, however, exhibited decreased fry survival in the first week following hatching (Hansen et al., 1974a).

STLCOPCB4012754

This study indicates that although Aroclor 1016 is readily accumulated by and is toxic to fry, juvenile and adult sheepshead minnows, it is about 100 times less toxic than Aroclor 1254 to sensitive fry. More important, concentrations of Aroclor 1016 in eggs as great as 77 µg/g apparently did not affect survival of embryos and fry for two weeks.

STLCOPCB4012755

REFERENCES

Brungs, W. A. and D. I. Mount. 1970. A water delivery system for small fish-holding tanks. Trans. Amer. Fish. Soc. 99(4):799-802.

Butler, P. A. 1973. Organochlorine residues in estuarine mollusks, 1965-1972. National Pesticide Monitoring Program. Pestic. Monit. J. 6(4):238-362.

Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor 1254®) in the water, sediment and biota of Escambia Bay, Florida. Bull. Environ. Contam. Toxicol. 5(2):171-180.

Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr. and P. D. Wilson. 1971. Chronic toxicity, uptake and retention of Aroclor® 1254 in two estuarine fishes. Bull. Environ. Contam. Toxicol. 6(2):113-119.

Hansen, David J., Steven C. Schimmel and Jerrold Forester. 1974a. Aroclor® 1254 in eggs of sheepshead minnows: Effect on fertilization success and survival of embryos and fry. Proc. S. E. Assoc. Game Fish. Comm. (In press).

Hansen, D. J., P. R. Parrish and J. Forester. 1974b. Aroclor® 1016: Toxicity to and uptake by estuarine animals. Environ. Res. (In press).

Litchfield, J. T., Jr. and F. Wilcoxon. 1949. A simplified method of evaluation dose-effect experiments. J. Pharmacol. Exp. Ther. 96(2):99-113.

Lowe, J. I., P. R. Parrish, J. M. Patrick, Jr. and J. Forester. 1972. Effects of the polychlorinated biphenyl Aroclor® 1254 on the American oyster Crassostrea virginica. Mar. Biol. (Berl.) 17(3): 209-214.

DSW 028794

STLCOPCB4012756

Nimmo, D. R., R. R. Blackman, A. J. Wilson, Jr. and J. Forester. 1971. Toxicity and distribution of Aroclor® 1254 in the pink shrimp, Penaeus duorarum. Mar. Biol. (Berl.) 11(3):191-197.

Nimmo, D. R. and L. H. Bahner. 1974a. Physiological consequences of polychlorinated biphenyl- and salinity-stress in penaeid shrimp. Symposium "Pollution and the physiological ecology of estuarine and coastal water organisms. (In press).

Nimmo, D. R., J. Forester, P. T. Heitmuller and G. H. Cook. 1974b. Accumulation of Aroclor® 1254 in grass shrimp (Palaemonetes pugio) in laboratory and field exposures. Bull. Environ. Contam. Toxicol. (In press).

Schimmel, Steven C., David J. Hansen and Jerrold Forester. 1974. Effects of Aroclor® 1254 on laboratory-reared embryos and fry of sheepshead minnows (Cyprinodon variegatus). Trans. Amer. Fish. Soc. (In press).

STLCOPCB4012757

Table I. -- Toxicity of Aroclor® 1016 to embryos and fry, juvenile and adult sheepshead minnows (Cyprinodon variegatus) exposed for 28 days in two separate experiments.

CONCENTRATION (µg/ℓ)		PERCENTAGE SURVIVING FOR 28 DAYS		
NOMINAL	MEASURED	EMBRYOS AND FRY	JUVENILES	ADULTS
EXPERIMENT 1				
CONTROL	ND[a/]	76	100	96
0.10	ND	76	100	96
0.32	ND	73	100	100
1.0	0.46	90	100	100
3.2	1.4	75	100	96
10.	5.5	61	100	84
EXPERIMENT 2				
CONTROL	ND	77	100	92
1.0	0.29	78	96	88
3.2	1.0	67	100	88
10.	3.4	71	96	88
32.	15.	14	12	8
100.	42.	0	0	0

a/ ND = not detectable; <0.2 µg/ℓ

DSW 028796

STLCOPCB4012758

Table II -- Nominal concentration (µg/ℓ) of Aroclor® 1016 lethal to 50 percent of the fry, juvenile, and adult sheepshead minnows (LC50) each week during a four-week bioassay. Ninety-five percent confidence limits are in parentheses.

STAGE	WEEK			
	1	2	3	4
Fry*	>100	31 (25-38)	24 (21-28)	21 (18-25)
Juveniles	>100	47 (35-62)	22 (17-28)	20 (15-26)
Adults	>100	42 (29-61)	28 (22-36)	19 (14-25)

*Bioassay began with eggs and embryos hatched from day four to day nine.

DSW 028797

STLCOPCB4012759

Table III -- Concentrations of Aroclor® 1016 in fry, juvenile and adult sheepshead minnows exposed for 28 days. Each sample consisted of a minimum of 14 fish. Concentration factors in parentheses.

CONCENTRATION IN WATER (µg/ℓ)		CONCENTRATION IN WHOLE FISH (µg/g wet weight)		
NOMINAL	MEASURED	FRY [a]	JUVENILES	ADULTS
EXPERIMENT 1				
CONTROL	ND[b]	ND[b]	ND	ND
0.10	ND	ND	2.3 (23,000)	0.84 (8,4
0.32	ND	0.81 (2,500)	8.9 (28,000)	1.5 (4,7
1.0	0.46	4.9 (4,900)	11. (11,000)	12. (12,0
3.2	1.4	22. (6,900)	79. (25,000)	46. (14,0
10.	5.5	38. (3,800)	230. (23,000)	100. (10,0
EXPERIMENT 2				
CONTROL	ND	ND	ND	ND
1.0	0.29	5.9 (5,900)	10. (10,000)	5.4 (5,4
3.2	1.0	26. (8,100)	54. (17,000)	22. (6,9
10.	3.4	57. (5,700)	220. (22,000)	110. (11,0
32.	15.	200. (6,200)	1100. (34,000)[c]	--
100.	42.	--	--	--

[a] Embryos and fry were exposed for 33 days.

[b] ND = not detectable; <0.2µg/ℓ in water, <0.2 µg/g in tissue.

[c] Sample consisted of three juvenile fish.

DSW 028798

STLCOPCB4012760

Table IV -- Success of fertilization of eggs from adult sheepshead minnows exposed to Aroclor® 1016 for four weeks, survival of embryos from fertile eggs until hatching and survival of hatched fry. Eggs are from five fish, 25 eggs each, per concentration. Percentages are in parentheses.

NOMINAL CONCENTRATION IN WATER (µg/ℓ)	CONCENTRATION IN EGGS (µg/g)	EGGS			FRY	
Adult Exposure	Average (range)	Tested	Fertile	Hatched	Week 1	Week 2
Control	ND[a]	125	119 (95)	82 (69)	81 (99)	60 (73)
1.0	4.2 (3.1-5.1)	124	120 (97)	76 (63)	71 (93)	64 (84)
3.2	17. (12-19)	125	122 (98)	102 (84)	99 (97)	91 (89)
10.	66. (51-77)	124	117 (94)	88 (75)	84 (96)	72 (82)

[a] ND = not detectable; 0.2 µg/g

DSM 028799

STLCOPCB4012761

(32)

Toxicity of Aroclors 1254 and 1016 to Salt Water Organisms in Flow-through Bioassays Exceeding 96-hour Duration.

Chemical	Organism	Duration (Days)	Measured Concentration (µg/ℓ) Significant Mortality	Measured Concentration (µg/ℓ) No Significant Mortality	Exhibit Number
Aroclor 1254	Blue crabs	20	–	3.5-4.2	1
	Pink shrimp	20	3.5-4.2	–	1
	Pink shrimp (juvenile)	15	0.94	0.57	5
	Pink shrimp	35	3.5	–	5
	Brown shrimp	30	3	–	24
	Grass shrimp	16	4.0	1.3	6
	Spot	20-45	5.0(±20%)	1.0(±20%)	26
	Pinfish	14-35	"	–	26
	Sheepshead minnows (embryos)	7	3.5	1.0	27
	" " (fry) a)	21	0.16	0.06	27
	" " (Juveniles)	21	3.5	1.0	27
	" " (adults)	21	–	3.5	27
	" " (adults)	28	5.6	1.1	28
	" " (reproduction)	28 b)	0.14	0.09	28
	" " (fry)	0	7.0 c)	5.4 c)	28
	Oysters d)	168-210	3.9	0.64	21
	Communities of Animals e)	120	0.6	<0.1	29
Aroclor 1016	Pinfish	42	13	7	19
	Sheepshead minnows (fry, juveniles and adults)	28	15	3.4	31

DSW 028800

a) Fry exposed for about 7 days as embryos.

b) Adults exposed for 28 days, eggs fertilized and placed in "clean" H_2O.

c) Concentration in eggs µg/g wet weight.

d) Growth was slowed significantly by 3.9 µg/ℓ but not by 0.64 µg/ℓ.

e) Community composition was altered significantly by 0.6 µg/ℓ but not by <0.1 µg/ℓ.

STLCOPCB4012762