# AROCLOR® 1254 IN EGGS OF SHEEPSHEAD MINNOWS: EFFECT ON FERTILIZATION SUCCESS AND SURVIVAL OF EMBRYOS AND FRY[1]

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## ABSTRACT

The effect of the polychlorinated biphenyl (PCB), Aroclor 1254, in eggs of the sheepshead minnow, *Cyprinodon variegatus*, on fertilization success and survival of embryos and fry was investigated. Adult fish were exposed for four weeks to 0.1, 0.32, 1.0, 3.2 or 10.0 ug/*l* of PCB, then injected twice with 50 IU of human chorionic gonadotrophin to stimulate egg production. The eggs were fertilized, placed in PCB-free flowing seawater and observed for mortality. Fertilization success was unimpaired by concentrations in eggs as high as 201 ug/g but survival of embryos and fry was reduced. Usually, fry from eggs containing 7.0 ug/g or more began dying 24-48 hours after hatching. If this PCB affects other species similarly, then populations of fish that presently have comparable concentrations in their eggs may be endangered.

## INTRODUCTION

Polychlorinated biphenyls (PCB's) have been found frequently in estuarine organisms from many states (Butler, 1973) and in an estuary near the Gulf Breeze Laboratory (Duke et al., 1970). PCB's in seawater are toxic to and accumulated by juvenile shrimp, crabs, oysters and fishes (Nimmo, et al., 1971; Lowe, et al., 1972 and Hansen, et al., 1971). The relationship between the amount accumulated by fish and subsequent effects is poorly understood. However, PCB's in eggs may decrease fertility and survival in early stages of embryonic development in Atlantic salmon, *Salmo salar* (Johannsson et al., 1970), and PCB's have been implicated in poor reproductive success of striped bass, *Morone saxatilis* (Anonymous, 1971). Because reproductive success with both fishes varied, the exact relationship of success to concentration of PCB in eggs remains unclear.

Our study was conducted to determine the effect of one PCB, Aroclor 1254, on fertilization success of eggs of sheepshead minnows, *Cyprinodon variegatus*, and on survival of embryos and fry. Aroclor 1254 was selected because we found eggs from striped bass that exhibited decreased reproductive success contained a PCB whose chormatograms closely resembled Aroclor 1254. Sheepshead minnows were selected because they can be readily exposed in the laboratory and reproductive success is excellent.

## MATERIALS AND METHODS

*Test fish*

Adult sheepshead minnows were seined from ponds on laboratory grounds and acclimated to laboratory conditions for four days before exposure. During

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acclimation, mortality was less than 1% and no abnormal behavior was observed. Females averaged 42.5 mm standard length, range 35-52 mm, and males averaged 42.8 mm, range 35-52 mm. During acclimation and exposure, fish were fed commercial fish food that contained no detectable PCB ( 0.01 ug/g).

*Adult exposure*

We exposed 20 female and 10 male fish in aquaria containing none, 0.1, 0.32, 1.0, or 3.2 ug/*l* of Aroclor 1254 and exposed 25 females and 15 males to 10 ug/*l* for four weeks in an intermittent-flow bioassay. The apparatus used was a modification of that of Brungs and Mount (1970). In our modification, Aroclor 1254 and carrier, polyethylene glycol 200, were injected into seawater each time the apparatus cycled. Each cycle siphoned water to six 80*l* test aquaria. The injection device was operated by a solenoid that raised a lever each cycle turning gears on six injectors and pushing the plungers of six 50cc syringes. Each of the approximately 150 daily cycles delivered 1.5*l* of filtered 30C seawater, 11 ug of carrier and appropriate amounts of PCB to each aquarium. Water and carrier without PCB were delivered to the control aquarium. Salinity of water averaged 17 o/oo, range 5 to 28 o/oo.

*Egg fertilization, embryo and fry survival*

The effect of Aroclor 1254 in eggs was determined by enhancing egg production in exposed fish by hormonal injection, fertilizing the eggs artificially and monitoring their development in flowing PCB-free seawater. Female sheepshead minnows were injected intraperitoneally with 50 I. U. human chorionic gonadotrophic hormone on exposure-days 25 and 27. On day 28, eggs were stripped manually from five females from each aquarium and those from each female placed in individual beakers containing 40 ml of filtered 30C seawater. Ninety-three of 96 females that survived produced eggs. Eggs from a female were fertilized with excised macerated testes from a male from the same aquarium. In addition, eggs from five control fish and two fish surviving exposure to 10 ug/*l* PCB were fertilized by males exposed to 1.0 ug/*l*. Twenty-five eggs from each fish were placed in Petri dishes to which a nine cm high collar of 500u nitex mesh was glued. Dishes were submerged 7 cm in the 80*l* aquarium which received approximately 225l of filtered PCB-free seawater per day; average salinity was 18 o/oo, range 10 - 27 o/oo. Success of fertilization was confirmed by checking microscopically for cleavage 1.5 hours after fertilization. Thereafter, dishes were checked daily to determine survival of embryos and fry. Dishes remained in the aquarium for 34 days. Fry were fed brine shrimp nauplii or dry commercial fish food daily.

*Chemical analyses*

Concentrations of Aroclor 1254 in water, eggs and fish were determined by electron capture gas-chromatography. Unfiltered water samples from each aquarium were analyzed weekly during the four-week exposures of adults. At the end of the adult exposure, concentrations were determined in the fertilized eggs from each fish and in surviving adult males and females. Also, fry that hatched from these eggs and survived for four weeks in PCB-free water were analyzed for Aroclor 1254 content. Analytical methods for water, eggs and fish were the same as those of Nimmo et al. (1971), except than an OV-101 column was used and all peak heights were summed for PCB quantification. Recovery efficiency of Aroclor 1254 exceeded 80%. Measured concentrations were not corrected for percentage recovery.

[2]Manufactured by George Frazer, 4528 Pitt Street, Duluth, Minn. 55804.

*Statistical analysis*

Probit analysis was used to determine whether increasing concentration of PCB in eggs increased the effect on fertilization success and on survival of embryos and fry. The $\chi^2$ test for independent samples was used to compare data for eggs from individual unexposed and exposed fish. Differences were considered real at $\alpha = 0.05$ for probit analysis and $\alpha = 0.01$ for $\chi^2$ tests.

## RESULTS AND DISCUSSION

Aroclor 1254 in water was toxic to and accumulated by adult sheepshead minnows exposed for four weeks (Table 1). Mortality of fish was negligible, except in the aquarium receiving 10 ug/*l*. Dying fish in this aquarium typically became lethargic, ceased feeding, and some developed fin rot. Fish accumulated the PCB in direct proportion to the concentration in the water and concentrations in fish ranged from 15,000 to 30,000 X the nominal concentration in the water. Concentrations in males and females were similar. Concentrations of the chemical in eggs from exposed adult fish were proportional to the concentrations in the fish and concentrations in female fish were 1.8 to 2.3 times greater than the concentrations in their eggs. The PCB exposure apparently did not alter the percentage of females producing eggs or their fecundity.

Fewer embryos and fry from eggs of exposed fish survived than did embryos and fry from eggs of control fish (Table 2). The percentage of the eggs fertilized was not affected, but survival of embryos to hatching was less in eggs from fish exposed to 10 ug/*l*. Survival rate of fry in the first week following hatching was less in eggs from fish exposed to 0.32 to 10.0 ug/*l* than in eggs from unexposed fish. The estimated LC50 was 6.1 ug/g; 95 percent confidence limit equals 3.5 to 11.8 ug/g. Fry typically began to die one or two days after hatching, about the time they started feeding. If fry survived the first week, there seemed to be no additional mortalities related to PCB during three weeks of additional observation. Concentrations of Aroclor 1254 in surviving fry were similar, 0.26-0.56 ug/g, and not proportional to concentrations in eggs.

Embryo survival decreased at the highest concentration of PCB in eggs and fry survival decreased with increasing concentration of PCB in eggs. The amount in eggs was critical because it was the sole source of PCB for the embryos and fry reared in PCB-free water. PCB in milt was probably not critical to fertility and survival because when eggs from control fish were fertilized with milt from either control or 1.0 ug/*l* exposed males, survival rate of embryos and fry was not altered (Table 2). Survival rate of fry hatched from eggs containing 7.0 ug/g or more of PCB was significantly less than the lowest survival rate of eggs from any of the five control fish (Table 3).

If the effect of PCB in eggs of other fishes is similar to that found with sheepshead minnows — and we have no data to support this view — then variations in published information concerning the chemicals relation to spawning success could be explained. Atlantic salmon eggs containing up to 1.9 ug/g of PCB had decreased fertility and survival of early embryos, but survival of late embryos and sac fry was unimpaired (Johannsson et al., 1970). Chesapeake Bay striped bass eggs containing PCB's had decreased fertility and survival of newly hatched fry (Anonymous, 1971). Our analysis of eggs from eleven striped bass from the Eastern shore of Chesapeake Bay showed that the eggs contained about 2.5 to 8.7 ug/g of a PCB resembling Aroclor 1254. Because concentrations of PCB in eggs of sheepshead minnows as high as 201 ug/g were not accompanied by decreased fertility and only minimal embryo mortality, it seems unlikely that decreased fertility and embryo survival in Atlantic salmon and striped bass could be related solely to PCB in their eggs. Diminished survival of newly hatched striped bass fry, however, could be PCB-related since concentrations in

their eggs were similar to those in sheepshead minnow eggs which produced fry whose survival was poor.

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Table 1. Toxicity and uptake of Aroclor® 1254 by adult sheepshead minnows (*Cyprinodon variegatus*) exposed for 28 days in an intermittent-flow bioassay. Thirty fish were tested per concentration. Residue analyses are for a minimum of 7 male and 17 female fish and eggs from 5 fish.

| TEST CONCENTRATION (ug/l) | | MORTALITY % | CONCENTRATION IN FISH (ug/g, wet weight) | | | FEMALES GRAVID % | AVERAGE FECUNDITY No. |
|---|---|---|---|---|---|---|---|
| Nominal | Measured | | Males | Females | Eggs | | |
| Control | ND* | 7 | 0.64 | 0.47 | 0.52 | 100 | 97 |
| 0.1 | 0.09 | 13 | 2.5 | 1.9 | 0.88 | 89 | 121 |
| 0.32 | 0.14 | 7 | 9.7 | 9.3 | 5.1 | 100 | 110 |
| 1.0 | 0.39 | 10 | — | 25. | 11. | 100 | 127 |
| 3.2 | 1.1 | 3 | 49. | 49. | 27. | 94 | 152 |
| 10.0 | 5.6 | 95 | — | — | 170.** | 100 | 138 |

*ND = not detectable, < 0.03 ug/l
**Eggs from two fish.

Table 2. Success of fertilization of eggs from sheepshead minnows exposed to Aroclor® 1254 for four weeks, survival of embryos from fertile eggs until hatching and survival of hatched fry. Eggs are from five fish per concentration (except two fish from 10 ug/*l*). Percentages are in parentheses.

| CONCENTRATION | | EGGS | | | FRY | |
|---|---|---|---|---|---|---|
| Adult Exposure | Eggs Average | Tested | Fertile | Hatched | Survival Week 1 | Survival Weeks 2,3,4 |
| (ug/*l*) | (ug/g) | | | | | |
| Control | 0.52 | 125 | 125 (100) | 116 (93) | 111 (95) | 106 (96) |
| 0.1 | 0.88 | 125 | 120 (96) | 106 (88) | 103 (97) | 98 (95) |
| 0.32 | 5.1 | 126 | 120 (95) | 107 (80) | 82 (77)* | 76 (93) |
| 1.0 | 11. | 126 | 121 (96) | 118 (98) | 31 (26)* | 26 (96) |
| 3.2 | 27. | 126 | 118 (94) | 100 (85) | 23 (23)* | 19 (83) |
| 10.0 | 170. | 50 | 46 (92) | 33 (72)* | 0 (0)* | 0 (--) |
| Control F and 1.0 ug/*l* M | -- | 128 | 119 (93) | 113 (95) | 112 (99) | 111 (99) |

*Significantly less than control hatching or one week fry survivals ($X^2$; $\alpha = 0.01$).

Table 3. Comparison of concentration of Aroclor® 1254 in eggs (wet weight) from sheepshead minnows exposed to the PCB for four weeks and success of fertilization of eggs and survival of embryos and fry.

| Concentration in Eggs | EGGS | | | FRY | | Adults from Aquaria |
|---|---|---|---|---|---|---|
| | Tested | Fertile | Hatched | Week 1 | Weeks 2,3,4 | |
| (ug/g) | | | | | | (ug/*l*) |
| 0.41 | 25 | 25 | 23 | 23 | 22 | Control |
| 0.44 | 25 | 25 | 24 | 19 | 19 | " |
| 0.45 | 25 | 25 | 25 | 25 | 24 | " |
| 0.53 | 25 | 25 | 24 | 24 | 23 | " |
| 0.57 | 25 | 24 | 19 | 18 | 18 | 0.1 |
| 0.76 | 25 | 25 | 20 | 20 | 18 | Control |
| 0.84 | 25 | 23 | 21 | 21 | 19 | 0.1 |
| 0.91 | 25 | 25 | 25 | 23 | 23 | " |
| 0.98 | 25 | 23 | 17 | 17 | 15 | " |
| 1.1 | 25 | 25 | 24 | 24 | 23 | " |
| 3.7 | 25 | 23 | 22 | 21 | 20 | 0.32 |
| 4.1 | 26 | 26 | 23 | 22 | 21 | " |
| 5.4 | 25 | 24 | 19 | 13 | 12 | " |
| 5.4 | 25 | 24 | 22 | 21 | 19 | " |
| 7.0 | 25 | 23 | 21 | 5 | 4 | " |
| 7.1 | 26 | 23 | 22 | 2 | 2 | 1.0 |
| 9.5 | 25 | 24 | 23 | 7 | 7 | " |
| 10.8 | 25 | 25 | 25 | 8 | 5 | " |
| 13.2 | 25 | 25 | 25 | 9 | 7 | " |
| 13.3 | 25 | 24 | 23 | 5 | 5 | " |
| 23.6 | 25 | 23 | 23 | 4 | 4 | 3.2 |
| 25.7 | 25 | 24 | 16 | 6 | 6 | " |
| 27.9 | 25 | 25 | 23 | 0 | 0 | " |
| 28.6 | 25 | 25 | 25 | 5 | 3 | " |
| 28.7 | 26 | 21 | 13 | 8 | 6 | " |
| 145. | 25 | 23 | 15 | 0 | 0 | 10.0 |
| 201. | 25 | 23 | 18 | 0 | 0 | " |