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Toxicity of the PCBs Aroclor 1254 and 1242 to Embryos and Larvae of the Mummichog, *Fundulus heteroclitus*

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PCBs as contaminants of the aquatic environment have aroused concern because of their toxicity, persistence, and tendency to bioaccumulate. Numerous studies of their effects on aquatic biota have been published. HALTER & JOHNSON (1974) found premature hatching of coho salmon (Oncorynchus kisutch) in 15 ug/L of Aroclor 1254. HANSEN et al. (1975) found that hatching time of Cyprinodon variegatus was unaffected by Aroclor 1016, and that eggs and larvae had comparable tolerances. However, SCHIMMEL et al. (1974) reported C. variegatus larvae to be more sensitive than embryos to Aroclor 1254, and MAUCK et al. (1978) reported that larvae of the brook trout (Salvelinus fontinalis) were also more sensitive than embryos. NEBEKER et al. (1974) found that embryos of the fathead minnow and flagfish were also more resistant to Aroclor 1242 than larvae of these species.

The mummichog, Fundulus heteroclitus, is an abundant estuarine species. It is easily maintained and its embryos have been staged. In previous studies we have described teratological effects of insecticides (WEIS & WEIS 1974), and of heavy metals (WEIS & WEIS 1977 a,b) on this species, and have noted that different females produced eggs with differing degrees of sensitivity. When subjected to the same concentration of methylmercury (MeHg) some batches were severely affected while others were only mildly affected (WEIS et al., in press).

In the present study we wished to learn the effects of the PCBs Aroclor 1242 and 1254 on mummichog embryos, and the degree of variation in susceptibility among eggs of different females. We also wished to see if the embryonic tolerance of a batch of embryos would be paralleled by larval tolerance, or by the embryonic MeHg tolerance, and whether prior exposure to PCB as embryos could alter the tolerance of the larvae.

MATERIALS AND METHODS

Adult fish were collected from Southampton, NY by seining. Eggs and sperm were stripped from fish into individual fingerbowls of filtered seawater (30 o/oo salinity). Toxicants were added after cleavage began, at which time eggs which failed to cleave were removed. The standard length of each female was recorded. Each batch of eggs was divided into a control group, a group treated with 0.05 mg/L MeHg (I.C.N. Pharmaceuticals, Plainview NY dissolved first in 0.2% $NaHCO_3$) and groups treated with Aroclor 1254

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or 1242 (Analabs Inc. North Haven CT) dissolved first in acetone for a stock solution of 10 mg/mL. This stock solution was dispersed in filtered sea water for an intermediate dilution of 0.1 mg/mL which was made up fresh daily. Previous work having shown that acetone had no gross effects on these embryos (WEIS & WEIS 1974); controls were untreated. All experimental groups contained 20 or more eggs. Solutions were changed daily for the first four days of development.

After one week of incubation at 24° C embryos were examined under a stereomicroscope for malformations. The MeHg response of each egg was evaluated by indices previously described (WEIS & WEIS 1977b, WEIS et al. in press) which rank the relative severity of defects in the head, heart, and skeletal system. Mean indices for each batch were calculated.

At the time of hatching, a retardation of hatch due to PCB was often noted. To stimulate hatching, both control and PCB-treated batches had water changed daily starting on day 12 of development. Clean water was used in all cases. PCB-treated batches were evaluated using a hatch ratio (HR) which is %hatch in PCB/% hatch in controls. This was calculated on the first day in which at least 50 % of the controls had hatched. The HR was generally below 1.0, reflecting retardation of hatching in PCB.

Larval susceptibility was determined by a short-term toxicity test with 5.0 mg/L PCB. Newly hatched fry from control groups and PCB-treated groups were separated into control and experimental dishes in 50 mL seawater. At least 10 experimental larvae were used in each dish. Water was changed and re-dosed daily for a week. The percent dead every 24 h was recorded. All larvae were unfed.

Spearman's Rank correlation coefficient (SIEGEL 1956) was performed to correlate the HR response of each batch of embryos to PCB with the indices of MeHg susceptibility, the standard length of the female, and the larval susceptibility of controls from that batch. The susceptibility of larvae which had previously been exposed as embryos was also compared with the susceptibility of larvae which had not received prior exposure.

RESULTS

The first group of experiments was done with Aroclor 1254. Eight batches of eggs were exposed to concentrations of 0.01 - 10.0 mg/L with no effect on embryonic development or hatching. Embryonic mortality was negligible, and some groups showed acceleration, some retardation, and some no effect on hatching. Larval tests for seven days likewise showed no larval mortality of former control eggs in concentrations up to 10.0 mg/L. However, in the four batches in which we tested larvae which had been pre-exposed to 10.0 mg/L as embryos, larval mortality (about 20 %) was noted after 72 h in 5.0 mg/L.

The second group of experiments was done with Aroclor 1242. Twenty-seven batches of eggs were exposed to 10.0 mg/L which also did not cause any malformations, but did cause a consistent retardation of hatching. The HR

varied from 0 (none hatched by the day 50% of controls had hatched) to 1.0 (equal percentages of controls and experimentals hatched). There was no correlation (by Spearman's test) of this PCB response with the indices of MeHg response. However, the HR was positively correlated with female standard length (Fig. 1). It can be seen that longer (and presumably older) females produced eggs that were less affected by the PCB. There was also a correlation of HR with fecundity (number of eggs in the batch) ($P < 0.01$) although this is probably a secondary relationship, since larger fish tend to produce more eggs.

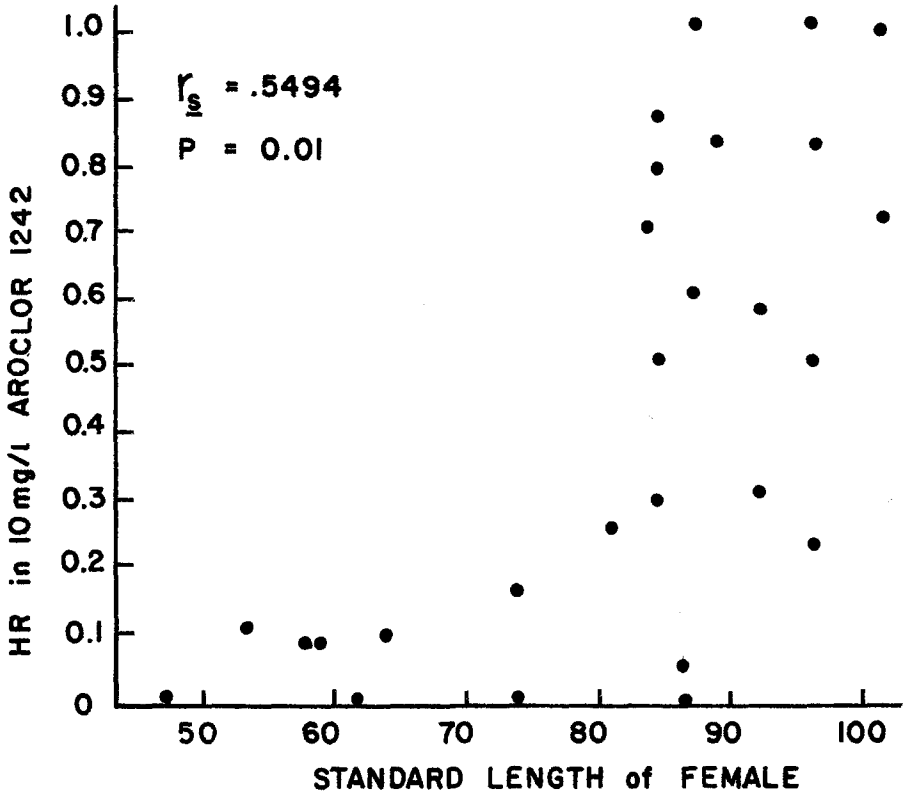

Figure 1. Relationship of Hatch Ratio (HR) of a batch of eggs exposed to 10 mg/L Aroclor 1242 to maternal length.

For the short-term larval toxicity test 5.0 mg/L was chosen as the standard dose, since this concentration did not kill all larvae immediately, nor did they all survive the 7-day period. The percentage dead at 72 h varied from 0 - 100%, and averaged 45 ± 11 percent among 17 batches of larvae that hatched from control groups of embryos. This larval tolerance was not correlated with the HR of the embryos, however. Larval mortality in 5.0mg/L was considerably higher among the 9 groups which had previously been exposed to 10.0 mg/L as embryos. In these groups, the percentage dead at 72 h was 11 % in one group and 100 % in all the others. Nine batches exposed to 1.0 mg/L as embryos showed an intermediate response. Fig. 2 shows the comparison of mortality among groups of larvae with no pre-exposure, pre-exposure to 1.0 mg/L, and pre-exposure to 10.0 mg/L as embryos. Larval deaths in pre-exposed groups tended to be precipitous, going from 10-20% dead at 48 h to 100% dead at 72 h. No mortality occurred in any control larvae, including in groups which had had prior exposure to PCB as embryos.

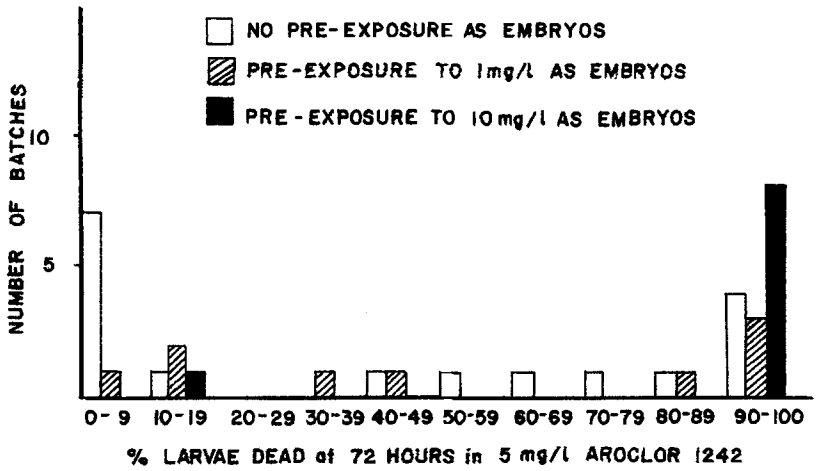

Figure 2. Larval mortality in 5.0 mg/L Aroclor 1242 in groups with no embryonic pre-exposure, pre-exposure to 1.0 mg/L, and pre-exposure to 10.0 mg/L.

DISCUSSION

Mummichog embryos proved to be highly resistant to the PCBs in these short-term tests. No teratological effects were noted in embryos in

up to 10 mg/L. It is likely that the actual concentrations were less than the nominal concentrations introduced. LAUGHLIN et al. (1977) and ROESIJADI et al. (1976) have shown actual concentrations to be considerably less than the nominal concentrations, and that a decrease of 30-60% took place after 24 h. ROESIJADI et al. (1976) attributed the rapid loss of PCB to volatilization, adsorption onto the glass, and uptake by organisms. The daily changing of our solutions helped to bring the level up, but we did not measure actual concentrations.

We found the larvae to be more sensitive to Aroclor 1242 than embryos. This is similar to the findings of SCHIMMEL et al. (1974) with Aroclor 1254 on Cyprinodon variegatus. The concentrations used by those investigators were considerably lower than ours, reflecting the long-term nature of their study, as compared to the short-term nature of ours, but also, probably, a greater tolerance on the part of F. heteroclitus than other species tested. HALTER & JOHNSON (1974) noted embryo mortality of coho salmon (Oncorynchus kisutch) in 15 ug/L of Aroclor 1254, and FREEMAN & IDLER (1975) noted great reduction in hatchability of brook trout (Salvelinus fontinalis) eggs in 0.2 mg/L Aroclor 1254. SCHIMMEL et al. (1974) noted decreased embryo survival of C. variegatus in 10 ug/L Aroclor 1254. These are all much lower concentrations than used in the present study.

Aroclor 1254 had an extremely low toxicity to F. heteroclitus in that (nominal) levels up to 10 mg/L produced no noticeable effects on embryonic development, hatching, or larval survival during a 7-day period (except for pre-exposed larvae). Other investigators have also noted differential toxicities of different PCBs. NEBEKER et al. (1974) noted that Aroclor 1254 was more toxic than Aroclor 1242 to the fathead minnow, Pimephales promelas (this being the reverse of our findings), but MAYER et al. (1977) found Aroclor 1242 to be more toxic than 1254 to cutthroat trout. DEFOE et al. (1978) found Aroclor 1260 to be more toxic than 1248 to P. promelas, and HANSEN et al. (1975) found Aroclor 1016 only 0.01 times as toxic as 1254 to C. variegatus.

The degree to which hatching was retarded by Aroclor 1242 was inversely correlated with the length of the female that produced the eggs. This is probably a reflection of the general phenomenon that older fish produce more successful eggs with greater hatchability (PITTMAN 1979). We have previously found embryonic Pb tolerance to be correlated with female length in F. heteroclitus (WEIS & WEIS, in press).

No correlation of PCB tolerance with MeHg tolerance was observed, showing that tolerance to one toxicant does not imply tolerance to others. This was previously noted with MeHg and Pb tolerance (WEIS & WEIS, in press).

The effects of PCB on larval survival indicate, as mentioned previously, greater susceptibility than the embryos to Aroclor 1242. Larval tolerance

was not correlated with embryonic tolerance, indicating that different mechanisms of toxicity are involved. Since embryonic tolerance was correlated with maternal length, it is likely that some maternally derived trait, probably chorionic permeability, is involved in conveying tolerance to the embryos. It is likely that the chorion protects embryos to differing degrees, but in all cases, the embryos would be exposed to less toxicant than the larvae. The differential susceptibility of different groups of larvae, on the other hand, must be due to differences among the larvae themselves.

Larvae which were pre-exposed as embryos were much more susceptible, indicating that the pre-exposure does not produce acclimation and then increased tolerance, as has been reported for a number of pollutants including heavy metals (DIXON & SPRAGUE 1981 a,b; LLOYD 1960) and pesticides (ORCIARI 1979). On the contrary, the pre-exposure to PCB served to weaken the larvae and make them more susceptible to further exposure. A similar increase in sensitivity was seen by DIXON & SPRAGUE (1981a) in cyanide-exposed rainbow trout (S. gairdneri), and by SWARTS et al. (1978) in sulfuric acid-exposed brook trout (S. fontinalis).

There is evidence that PCBs have cumulative, slow acting effects. MAUCK et al. (1978) have noted long-term effects on brook trout (S. fontinalis) larvae, showing that levels of Aroclor 1254 which had no effect on hatchability, hatching time and sac fry survival, did finally cause fry mortality after 48 days. Similar slow cumulative effects of Aroclor 1016 on pinfish (Lagodon rhomboides) were noted by HANSEN et al. (1974) and on C. variegatus by HANSEN et al. (1975). NIMMO et al. (1975), after two-week tests of Aroclor 1254 on several species of shrimp, long nose killifish (F. similis), and spot (Leiostomus xanthurus), concluded that acute tests did not show the true sensitivity of these species. In comparison to 48 h tests, those lasting two weeks showed a 100-fold increase in toxicity.

Although we have not done long-term, low dose experiments, our data showing increased larval susceptibility after embryonic pre-exposure, and the precipitous nature of the larval deaths indicate that a similar cumulative process is occurring in this species. It is likely that longer term larval tests would have revealed significant mortality at much lower dose levels.

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