Bull. Environm. Contam. Toxicol. 25, 744-750 (1980)

Inhibition by the Polychlorinated Biphenyl Aroclor 1242 of Limb Regeneration in the Fiddler Crab, *Uca pugilator*, in Different Salinities from which Different Numbers of Limbs Have Been Removed

Sue W. Fingerman and Milton Fingerman
Department of Biology, Tulane University, New Orleans, LA 70118

Although several environmental pollutants have been shown to affect development, growth, and limb regeneration in different species of crabs (WEIS and MANTEL 1976; FINGERMAN and FINGERMAN 1977, 1979a,b; WEIS 1978; LAUGHLIN and NEFF 1979), the only published reports on the effects of polychlorinated biphenyls (PCBs) on any crab, are those of FINGERMAN and FINGERMAN (1977, 1978, 1979a,b) which dealt with the effects of the PCB preparation Aroclor 1242 on color changes, the effects of the Aroclors 1242 and 1254 on molting and limb regeneration, and the work of NAGABHUSHANAM et al. (1979) dealing with the effects of Aroclor 1242 on the quantity of neurosecretory material in the medulla terminalis X-organ of the fiddler crab, Uca pugilator, having been used in all these PCB studies on crabs. Most pertinent to the present investigation are the reports by FINGERMAN and FINGERMAN (1977, 1979b) who found that low concentrations of PCBs greatly inhibit molting and the rate of regeneration of missing limbs by Uca pugilator. Because PCBs have such profound inhibitory effects on molting and limb bud regeneration in fiddler crabs and because crabs are constantly exposed to PCBs under varying environmental conditions, investigating the effects of PCBs on the rate of limb regeneration in crabs kept in different salinities of sea water as well as determining whether the degree of inhibition of limb bud regeneration exerted by PCBs is related to the number of legs lost seemed worthwhile.

MATERIALS AND METHODS

Mature female fiddler crabs, Uca pugilator, collected in the area of Panacea, FL, were obtained from the Gulf Specimen Company. The salinity of the water where the crabs were collected was 20 parts per thousand (ppt). On the day after a shipment of crabs was received in the laboratory, intact intermolt females having a carapace width of 1.4-1.7 mm were selected from the stock group for use in the experiments. The crabs used in the experiments were then placed individually into numbered containers covered with translucent lids and kept at 25°C under constant illumination. Inside the cups the light intensity was 275 lux. The fluid in the individual containers was about 15 mm deep. The crabs were fed uncooked oatmeal twice each week. The solutions in each container was changed after the crabs had been allowed to feed for approxi-

0007-4861/80/0025-0744 $01.40

mately three hours.

Different numbers of walking legs were removed, depending on the experiment. However, regardless of the number removed only the rate of regeneration of the first walking leg on the right side was observed. Limb removal was accomplished by inducing the crabs to autotomize the limb when the merus was pinched with forceps. A dissecting microscope fitted with an ocular micrometer was used to measure the regenerating first leg. The measurements were used to calculate R (regeneration) values (BLISS 1956) which are a measure of the rate of regeneration of the limb:

$$R \text{ value} = \frac{\text{length of limb bud in mm}}{\text{carapace width in mm}} \times 100$$

The R value for each crab was determined once a week. Student's t test was used to determine the statistical significance of the difference between means.

Aroclor 1242 (Monsanto Lot Number G266K) was the PCB preparation used. The Aroclor was first dissolved in ethanol or acetone and the solution was ultimately diluted 1:1000 in the appropriate concentration of artificial sea water (Instant Ocean, Aquarium Systems, Inc., Eastlake, Ohio) to provide the desired final concentration of 0.0008% of the PCB in sea water containing 0.1% acetone or 0.1% ethanol. Control crabs were kept in artificial sea water containing 0.1% acetone or 0.1% ethanol alone, the same concentration as in the sea water with PCBs.

EXPERIMENTS AND RESULTS

The object of the first set of experiments to be described was to determine (1) the effects of different salinities on the rate of leg regeneration in the fiddler crab and (2) the effect of the PCB, Aroclor 1242, on the rate of leg regeneration at different salinities. The first experiment of this set was started on April 8, 1978, and repeated beginning May 5, 1978. On day 1 of each experiment the four walking legs on the right side were removed from each crab. The reason for removing four walking legs was because removal of this number promotes precocious ecdysis in a high percentage of the crabs (FINGERMAN and FINGERMAN 1974). In each experiment one group of crabs was exposed to 17 ppt salinity + PCB, a second group to 34 ppt salinity + PCB, and a third group to 43 ppt salinity + PCB. The Aroclor 1242 was dissolved initially in ethanol. Three additional groups of crabs which served as the controls were exposed to the three salinities, 17 ppt, 34 ppt, and 43 ppt. One hundred percent sea water has a salinity of 34 ppt. The total number of crabs used in the two experiments of this set was 75 in each of the control groups, 141 in the 17 ppt salinity + PCB group, 151 in the 34 ppt salinity + PCB group, and 151 in the 43 ppt salinity + PCB group. R values were determined on days 14, 21, and 28 or until ecdysis had occurred. The data from the two experiments were averaged together

and the means are presented in TABLE 1.

Comparison of the three control groups (43, 34, and 17 ppt) reveals that on the fourteenth day the length of the regenerate of the 43 ppt control crabs was significantly smaller than in the 17 and 34 ppt control groups. But there was no significant difference among the three groups after 14 days.

Comparison of the control groups with the corresponding PCB groups reveals that at all times the limbs of the crabs exposed to PCB were significantly smaller than those of the corresponding controls. Furthermore, by the end of the experiment the mean R values for the limbs of the crabs in the 17 and 43 ppt salinity + PCB solutions were significantly less than those of the crabs in the 34 ppt salinity + PCB solution.

The object of the second set of experiments was (1) to determine if the degree of inhibition of limb regeneration caused by Aroclor 1242 might be related to the number of legs removed, (2) to compare the rate of regeneration of the first walking leg in crabs with two or six legs removed, and (3) to see whether removing four additional legs 14 days after two legs had been removed would cause accelerated regeneration if the rate was less in the crabs with two legs off than in those from which six legs had been removed. Removal of two legs was decided upon because there is a low rate of ecdysis in crabs missing two legs whereas removal of six legs gives a rapid rate of ecdysis (FINGERMAN and FINGERMAN 1974). The first experiment of this set was begun on June 21, 1978 and was repeated three times, beginning on July 7, 1978; July 12, 1979; and May 8, 1980. The Aroclor 1242 was dissolved in acetone; control groups received an equivalent amount of acetone in sea water. On day 1 of each experiment, the crabs to be used were divided into two groups. One group had the first two walking legs on the right side removed and one group had the four walking legs on the right side plus the first two walking legs on the left side removed. Then the two groups were divided again, one portion of each of the original groups then being exposed to the PCB- solution and were designated PCB-2 and PCB-6. The two remaining portions from each of the original two groups were exposed to the control solution and were designated control-2 and control-6. The number (as in PCB-2) refers to the numbers of legs that were removed. For the four experiments of this set a total of 200 individuals was used for the control-2 crabs, 100 for the control-6 crabs, 210 for the PCB-2 crabs, and 105 for the PCB-6 crabs. The rate of regeneration of the first right walking leg was determined on days 14, 21, and 28 or until ecdysis had occurred.

Additionally, after the limb buds had been measured on day 14, the -2 groups (control and experimental) were further divided. Some of the crabs were kept as they were (control-2 and PCB-2) and from the rest 4 more legs were removed, the posterior right walking legs and two anterior left walking legs, and these crabs were designated control-2-4 and PCB-2-4. Additional legs were not removed from the -6 legs groups. Measurements of limb bud growth

TABLE 1

Average R values (mean ± S.E. (Number)) of control and PCB-exposed female fiddler crabs in different salinities

Salinity	Control or PCB	14 days	21 days	28 days
17 ppt	Control	4.38 ± 0.23 (67)	8.52 ± 0.42 (65)	12.13 ± 0.54 (62)
34 ppt	Control	4.29 ± 0.22 (67)	8.45 ± 0.39 (65)	12.29 ± 0.60 (65)
43 ppt	Control	3.34 ± 0.20 (62)	7.42 ± 0.43 (60)	11.26 ± 0.64 (58)
17 ppt	PCB	1.97 ± 0.30 (54)	3.67 ± 0.55 (33)	3.58 ± 0.76 (23)
34 ppt	PCB	2.05 ± 0.29 (62)	4.91 ± 0.71 (47)	8.09 ± 1.11 (35)
43 ppt	PCB	1.10 ± 0.27 (60)	2.67 ± 0.59 (36)	3.85 ± 1.04 (23)

of the first right walking leg of all of the crabs were continued for two more weeks. The data from the experiments were averaged and the results are presented in TABLE 2.

The R values of the control and PCB crabs that had only two legs removed or two followed by the removal of four more were significantly less than those of the corresponding groups of control and PCB crabs that had six legs removed on day 1. Furthermore, removal of four additional legs did not produce a significant increase in the rate of limb regeneration. Also, as expected at all times the limbs of the PCB-exposed crabs grew at a significantly lower rate than did those of the corresponding controls.

DISCUSSION

The results obtained in the first set of experiments showed that by days 21 and 28 differing salinities had no effect on the rate of regeneration of the first walking leg of the control crabs in the three different salinities (TABLE 1). Only on day 14 was there a significant difference among them, the crabs in 43 ppt sea water showing a reduced growth at that time. However, among the PCB-exposed crabs inthe different salinities not only was regeneration greatly reduced in all the PCB groups, but the inhibitory effect of the PCB was by the end of the experiment significantly greater in the highest and lowest salinities tested. In the second set of experiments in this study, comparison of the R values for the PCB-exposed and control groups reveals that additional removal of legs after 14 days does not affect the rate of regeneration. Presumably, the rate of regeneration of a limb is set during the first 14 days of regeneration and the subsequent removal of additional limbs has no effect on the rate once established. The data reveal again that Aroclor 1242 inhibits limb regeneration. Furthermore, TABLE 2 reveals that the first walking leg regenerates at a faster rate both in PCB-exposed and control crabs from which six legs were removed than in the corresponding groups of crabs that lost only two. This increased rate has as more legs are lost obvious survival value. Although the results presented here and by FINGERMAN and FINGERMAN (1979b) show that Aroclor 1242 inhibits limb regeneration, not all pollutants have this effect. DDT (10 ppb), for example, accelerated limb regeneration in fiddler crabs (WEIS and MANTEL 1976). But like Aroclor 1242, methylmercury and cadmium retarded limb regeneration and ecdysis in fiddler crabs (WEIS 1978). The cadmium had a greater effect in reduced salinities. Similarly, herein, Aroclor 1242 was more inhibitory in dilute and concentrated sea water (TABLE 1). MCKENNEY and NEFF (1979) found that both salinity and temperature individually modify the developmental rates of the grass shrimp, Palaemonetes pugio. Outside the 18 to 23 ppt salinity, both higher and lower salinities and temperature retarded development. Their data indicate a significant salinity-temperature interaction. There was a greater retardation of rates in extreme salinities at lower temperatures, with low salinity retarding developmental rates slightly more at higher than at lower temperatures. LAUGHLIN and

TABLE 2

Average R values (mean ± S. E. (Number)) of control and PCB-exposed female fiddler crabs missing different numbers of legs

	DAYS		
	14	21	28
Control-2	2.29 ± 0.15 (163)	5.08 ± 0.27 (99)	6.59 ± 0.60 (96)
Control-2-4		5.07 ± 0.44 (43)	8.32 ± 1.52 (35)
Control-6	3.94 ± 0.56 (43)	9.68 ± 1.04 (30)	14.90 ± 0.75 (20)
PCB-2	0.97 ± 0.14 (112)	2.61 ± 0.46 (24)	4.02 ± 0.42 (20)
PCB-2-4		2.87 ± 0.45 (24)	4.02 ± 0.88 (10)
PCB-6	2.27 ± 0.37 (23)	5.77 ± 1.03 (14)	10.67 ± 2.63 (7)

NEFF (1979) found that the polycyclic aromatic hydrocarbons phenanthrene and naphthalene significantly affected the development of larval mud crabs, Rhithropanopeus harrisii. Phenanthrene-exposed larvae had a decreased developmental rate, but naphthalene-exposed larvae developed faster than the controls. Low level concentrations of the Aroclors 1242 and 1254 decreased limb bud growth in fiddler crabs, the effect being more pronounced at the time when molting activity in the crab population is normally high (FINGERMAN and FINGERMAN 1979b). There appears to be optimal salinity and temperature conditions for various species of crabs, so in considering the effects of sublethal concentrations of any pollutant it should be taken into account that other environmental factors are also influencing the potency of the pollutant.

REFERENCES

BLISS, D. E.: In: Bertil Hanström, zoological papers in honour of his sixty-fifth birthday, November 20, 1956, Wingstrand, K. G., ed. Lund: Zoological Institute 1956.

FINGERMAN, M., and S. W. FINGERMAN: Zool. Jb. Physiol. 78, 301 (1974).

FINGERMAN, S. W., and M. FINGERMAN: Bull. Environ. Contam. Toxicol. 18, 138 (1977).

FINGERMAN, S. W., and M. FINGERMAN: Mar. Biol. 50, 37 (1978).

FINGERMAN, S. W., and M. FINGERMAN: Bull. Environ. Contam. Toxicol. 21, 352 (1979a).

FINGERMAN, S. W., and M. FINGERMAN: Vie Milieu 29, 69 (1979b).

LAUGHLIN, R. B. JR., and J. M. NEFF: Mar. Biol. 53, 281 (1979).

MCKENNEY, C. L., JR., and J. M. NEFF: Mar. Biol. 52, 177 (1979).

NAGABHUSHANAM, R., S. W. FINGERMAN, and M. FINGERMAN: Experientia 35, 1083 (1979).

WEIS, J. S.: Mar. Biol. 49, 119 (1978).

WEIS, J. S., and L. H. MANTEL: Estuarine and Coastal Mar. Sci. 4, 461 (1976).