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Comparison of the Effects of Fourteen-Day and Chronic Exposures to a Polychlorinated Biphenyl, Aroclor 1242, on Molting of the Fiddler Crab, *Uca pugilator*

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Several species of crustaceans, including the fiddler crab, Uca pugilator, have been found to accumulate polychlorinated biphenyls (PCBs) from their environment (NIMMO et al. 1971a,b; 1974). But there have been few studies of possible physiological effects of these compounds, which are so extremely persistent in the environment, using crustaceans. NIMMO and BAHNER (1974) did find, however, that the PCB, Aroclor 1254, caused a significant decrease in the total ion concentration of the blood of brown shrimp, Penaeus aztecus. Grass shrimp, Palaemonetes pugio, exposed to Aroclor 1254 exhibited reduced water permeability, but this PCB did not cause an alteration in the osmotic concentration of the blood (ROESIJADI et al. 1976b). Also, Aroclor 1254 had no significant effect on the total free amino acid level in abdominal muscle of the grass shrimp, indicating thereby that PCBs do not affect intracellular osmoregulation to any great extent in this animal (ROESIJADI et al. 1976a). More closely related to the study described below is the report of FINGERMAN and FINGERMAN (1977) that another PCB, Aroclor 1242, inhibits molting of the fiddler crab, Uca pugilator.

The experiments described below were designed to extend the observations of FINGERMAN and FINGERMAN (1977). More specifically the object of the present experiments was to determine whether the inhibitory effect of Aroclor 1242 on molting of the fiddler crab, Uca pugilator, can be at least partially reversed by returning the crabs to sea water that did not contain this pollutant after they had been exposed to it for 14 days.

MATERIALS AND METHODS

Female fiddler crabs, Uca pugilator, obtained from a commercial dealer in Panacea, FL, were used. The crabs were intermolt specimens with a carapace width of 14-17 mm. They were kept throughout the experiment in individual styrofoam cups with translucent covers at 24°C under constant illumination. The light intensity inside the covered cups was 376 lux. The crabs were fed uncooked oatmeal twice weekly and the medium in which they were kept was changed after they had been allowed to feed for approximately 4 h. The fluid in each cup was about 15 mm deep.

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The PCB used in these experiments was Aroclor 1242 (Monsanto Lot Number GZ66K). It was first dissolved in acetone (8 mg/mL) and diluted 1:1000 with artificial sea water (Instant Ocean, Aquarium Systems) to provide the desired final concentration. Control crabs were exposed to artificial sea water containing 0.1% acetone, the same concentration as in the PCB-containing sea water.

Increased molting activity was induced by removal of both eyestalks (ZELENY 1905). This procedure eliminates the medulla X-organ-sinus gland complex which is the major source of molt-inhibiting hormone (BROWN and CUNNINGHAM 1939, PASSANO 1953). The eyestalk stubs were cauterized by use of an electric cautery to minimize bleeding. The incidence of molting in the laboratory among intact fiddler crabs is ordinarily quite low (FINGERMAN and FINGERMAN 1976). The crabs were observed daily to determine the incidence of ecdysis, the shedding of the old cuticle.

The data from the experiments described below are presented as the cumulative percentages of the crabs that had undergone ecdysis since the start of each experiment. These experiments were calculated daily by dividing the total number of crabs in each group that had undergone ecdysis through that day by the number of crabs that had undergone ecdysis plus the number alive that had not yet undergone ecdysis, and then multiplying the resulting ratio by 100. Each group consisted at the outset, Day 1, of 65 crabs. The experiments were performed twice. The averaged data from the two experiments of each set were used in the preparation of the figures presented below.

EXPERIMENTS AND RESULTS

The first experiment, to be described below, was started on September 9, 1976, and repeated beginning on October 6, 1976. It was designed to compare the rates of ecdysis of (a) crabs kept in Aroclor 1242-sea water throughout the experiment, (b) crabs transferred from Aroclor 1242-sea water into acetone-sea water after having been exposed to the PCB for the first 14 days of the experiment, and (c) crabs that had been in acetone-sea water from Day 1. The crabs in all three groups received a molt-promoting stimulus in the form of eyestalk removal on Day 15, the day one of the three groups was transferred from the PCB solution to acetone sea water. Not only was this one group taken from the Aroclor 1242-sea water but the cups in which they had been kept were replaced with new ones.

The averaged results from these two experiments are presented in Figure 1. As expected, the crabs that had been in acetone-sea water throughout the experiment underwent ecdysis at a rapid rate whereas those that had been in Aroclor 1242-sea water continuously since Day 1 underwent ecdysis at a very reduced

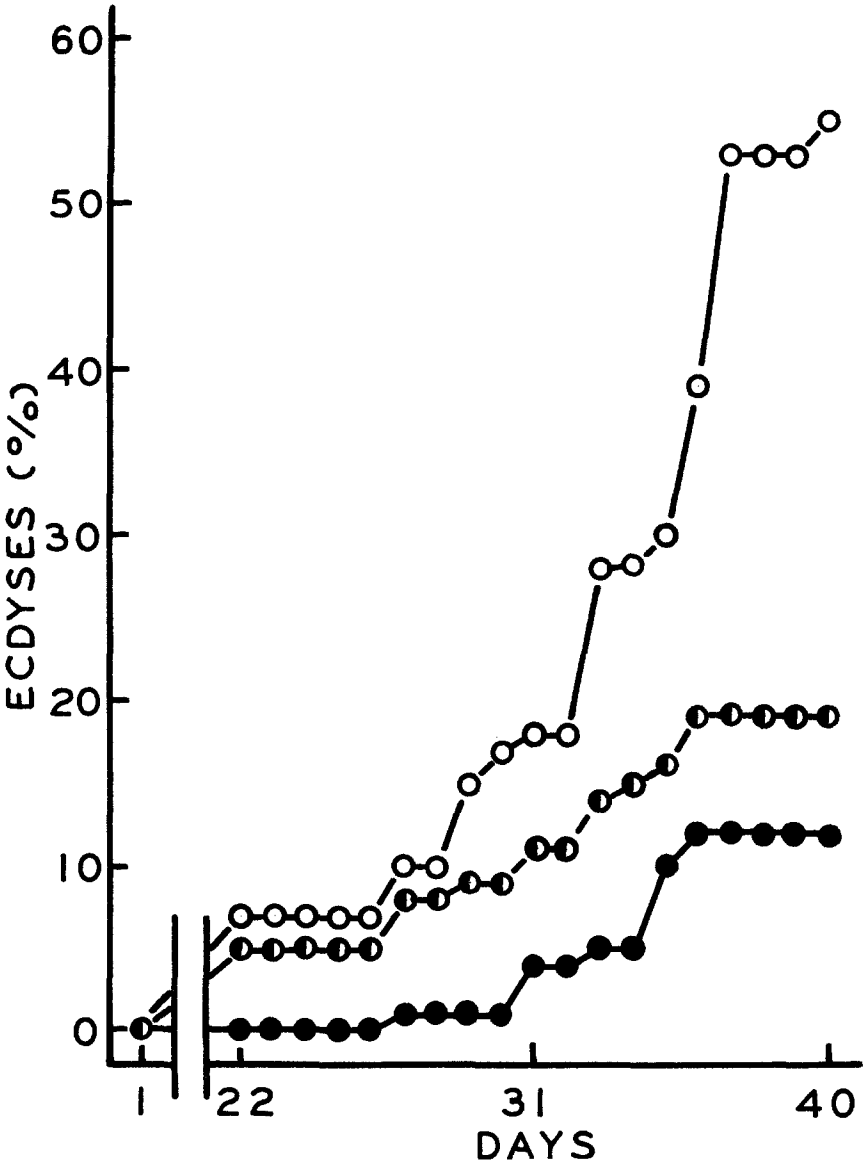

Figure 1. Relationship between rates of ecdysis and time in days. On Day 1 two groups of crabs (filled and half-filled circles) were exposed to Aroclor 1242. A third group (empty circles) served as the controls. On Day 15 the eyestalks were removed from the crabs in all three groups and one of the groups in Aroclor 1242 (half-filled circles) was transferred to acetone-sea water.

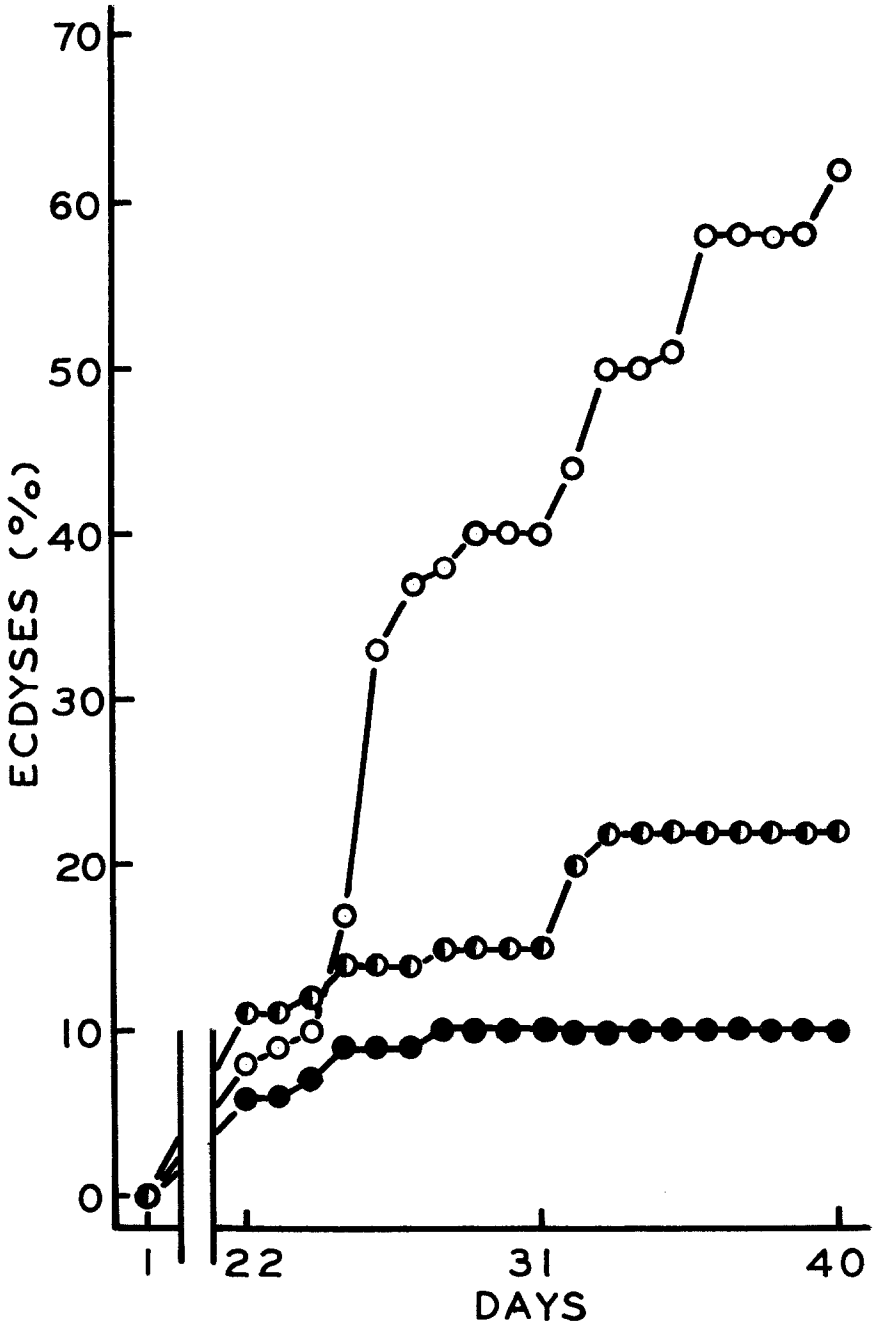

Figure 2. Relationship between rates of ecdysis and time in days. On Day 1 the eyestalks were removed from three groups of crabs and two of the groups (filled and half-filled circles were exposed to Aroclor 1242. The third group served as the control (empty circles). On Day 15, one of the groups in Aroclor 1242 (half-filled circles) was transferred to acetone-sea water.

rate. The crabs that had been taken from the Aroclor 1242-sea water and put into acetone-sea water on Day 15 underwent ecdysis at a slightly accelerated rate (7% higher) as compared with the crabs exposed to the PCB throughout the experiment, but still much more slowly than the controls. Presumably, the crabs that had been transferred from the PCB to acetone-sea water had absorbed sufficient Aroclor 1242 between Days 1 and 15 to inhibit ecdysis greatly even though from the time they were given the molt-promoting stimulus (eyestalk removal) until the end of the experiment they were no longer exposed to the pollutant.

The aim of the second set of experiments which was performed first beginning June 2, 1977, and a second time starting June 22, 1977, was a variation of the first experimental protocol described above. In this second series both eyestalks were removed on Day 1 from all the crabs instead of on Day 15 as was previously. In all other respects both experiments were the same. Once again (Fig. 2) the crabs that had been in acetone-sea water throughout underwent ecdysis at a high rate while those that had been continuously in Aroclor 1242-sea water underwent ecdysis at a very low rate. Furthermore, just as in Figure 1, the crabs that had been in Aroclor 1242 only for 14 days showed a diminished but greater rate of ecdysis (12% higher) than did the crabs that had been in the PCB throughout each experiment.

DISCUSSION

The average percentage of the control crabs that underwent ecdysis by the time each of the experiments was terminated (Day 40) was 8% greater among the crabs whose eyestalks had been removed on Day 1 (Fig. 2) than on Day 15 (Fig. 1). This difference was presumably due to the greater length of time these crabs had to undergo ecdysis after their eyestalks had been removed. There was very little difference in the percentages among the other corresponding groups of crabs, 2% for the crabs continuously exposed to the PCB and 3% for those that had been in the PCB for only the first 14 days of the experiment. Apparently, the crabs that had been in the PCB for only 14 days accumulated enough of it (presumably through their gills and while feeding) during that time to inhibit ecdysis greatly after they were no longer exposed to it. Furthermore, providing the molt-promoting stimulus (eyestalk removal) on Day 1, the same day the crabs were put in the PCB, to crabs that were to be exposed to it for only the first 14 days of the experiment did not overcome to any large degree the inhibitory action of the PCB. In fact, whether the molt-promoting stimulus was applied the day the crabs were placed in the PCB for 14 days or after they had been in it for 14 days made very little difference in the subsequent rate of ecdysis of the crabs. By the end of each experiment (Day 40) in which the eyestalks were removed on Day 15 (Fig. 1) an average of only 7% more of the crabs that were transferred to the acetone-sea water

underwent ecdysis than did crabs kept in the PCB for the entire experiment. Furthermore, by the time the experiments in which the eyestalks were removed on Day 1 were terminated (Fig. 2), only 12% more of the crabs that had spent only the first 14 days in the PCB underwent ecdysis than did the crabs kept in the PCB for the entire 40 days. It appears that once the crabs had been exposed for 14 days to the PCB concentration used herein they accumulated enough of it to have a long-lasting inhibitory effect on molting.

The results presented in Figures 1 and 2 are consistent with the findings of FINGERMAN and FINGERMAN (1977) who first reported that Aroclor 1242 has an inhibitory effect on molting in the fiddler crab. The results presented herein suggest a serious hazard for the survival of the fiddler crab if this persistent pollutant should continue to accumulate in the environment.

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