Effects of a Polychlorinated Biphenyl and a Polychlorinated Dibenzofuran on Molting of the Fiddler Crab, *Uca pugilator*

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Several species of aquatic crustaceans have been found to accumulate polychlorinated biphenyls (PCBs) from their environment. Specifically, NIMMO et al. (1971b) showed that the fiddler crab, Uca pugilator, accumulates the PCB, Aroclor 1254, from contaminated sediments. However, there are no published reports on the possible effects of PCBs on the physiology of the fiddler crab.

We undertook a series of experiments to determine the effect of the PCB, Aroclor 1242, on the rate of molting of the fiddler crab, Uca pugilator. Fairly recently, however, polychlorinated dibenzofurans (PCDFs) have not only been shown to be impurities in PCBs, but also to exceed the toxicity of PCBs in chick embryo assays by approximately four to six orders of magnitude (VOS et al., 1970; BOWES et al., 1975a,b). Consequently, reported effects of PCBs, as in mortality studies such as that of NIMMO et al. (1971a) with the pink shrimp, Penaeus duorarum, may have been due in large measure to contaminating PCDFs. Therefore, in the present investigation experiments were also performed to determine the effect of a PCDF on the rate of molting, the PCDF concentration used being that found in Aroclors by BOWES et al. (1975b). Use of such a PCDF concentration would allow us to determine whether any effects on molting were due to Aroclor 1242 or to perhaps only PCDF contamination of the Aroclor.

Materials and Methods

Female fiddler crabs, Uca pugilator, from Panacea, FL, were used. The crabs were intermolt individuals, having a carapace width 14-16 mm, and were intact at the outset. They were kept throughout each 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 twice weekly, after they had been allowed to feed for approximately four hours. The fluid level in the individual containers was about 15 mm deep. The crabs were induced to undergo ecdysis in two ways: (1) by removing both eyestalks (BROWN and CUNNINGHAM, 1939) and (2) by removing four walking legs (ZELENY, 1905; SKINNER and GRAHAM, 1972; FINGERMAN and FINGERMAN, 1974). These methods to induce molting

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Bulletin of Environmental Contamination & Toxicology,
Vol. 18, No. 2 © 1977 by Springer-Verlag New York Inc.

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activity were used because the rate of ecdysis of intact crabs in the laboratory is ordinarily very low (FINGERMAN and FINGERMAN, 1974, 1976). Limb removal was accomplished by inducing the crab to autotomize the limb when the merus of the limb was pinched.

The data from the experiments described below are presented as the cumulative percentages of the crabs that had undergone ecdysis since the start of the experiment. These percentages were calculated day by day by dividing the cumulative number of crabs that had undergone ecdysis by the total 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 experiment was performed twice. Averaged data from the two experiments of each set were used in the preparation of the figures presented below.

The PCB chosen for use in these experiments was Aroclor 1242 (Monsanto Lot Number 30016); the PCDF was 1,2,3,4,5,6,7,8-octachlorodibenzofuran (OCDF) (Analabs Lot Number 001). Both substances were first dissolved in acetone and ultimately diluted to the desired concentration in artificial sea water (Instant Ocean, Aquarium Systems, Inc.). The final Aroclor 1242 concentration was 8×10^{-4}% in sea water containing 0.1% acetone. The final OCDF concentration was 16×10^{-10}%. This OCDF concentration was used because 2 µg PCDF per gram of PCB was the highest PCDF concentration BOWES et al. (1975) found in any of the Aroclors they tested. The control crabs were kept in artificial sea water containing the same acetone concentration as in the sea water containing the PCB and OCDF.

Experiments and Results

The object of the first experiment, started July 22, 1975, and repeated beginning September 17, 1975, was to determine the effect of Aroclor 1242 on the rate of ecdysis of the fiddler crab. In each experiment four groups of 50 crabs each were used. From each crab in one group the four walking legs on its right side were removed. These crabs were then placed into cups containing Aroclor 1242. A comparable group lacking four legs served as controls. Both eyestalks were then removed from the members of the two remaining groups, one then being exposed to the Aroclor and the other serving as the control.

The eyestalkless control crabs and those lacking four walking legs that served as controls underwent ecdysis at a rapid rate (Figure 1). However, the groups that were in the Aroclor underwent ecdysis at a much slower rate than did the respective control groups. In fact, none of the eyestalkless crabs in the Aroclor underwent ecdysis during either experiment. In both Figures 1 and 2, Day 0 represents the day the experiment was started.

The object of the next set of experiments was to determine whether the decreased rate of ecdysis of the crabs kept in Aroclor 1242 (Figure 1) may have been due in any degree to a PCDF contaminant. Consequently, crabs lacking four walking legs and eyestalkless crabs were exposed to OCDF instead of Aroclor 1242 and

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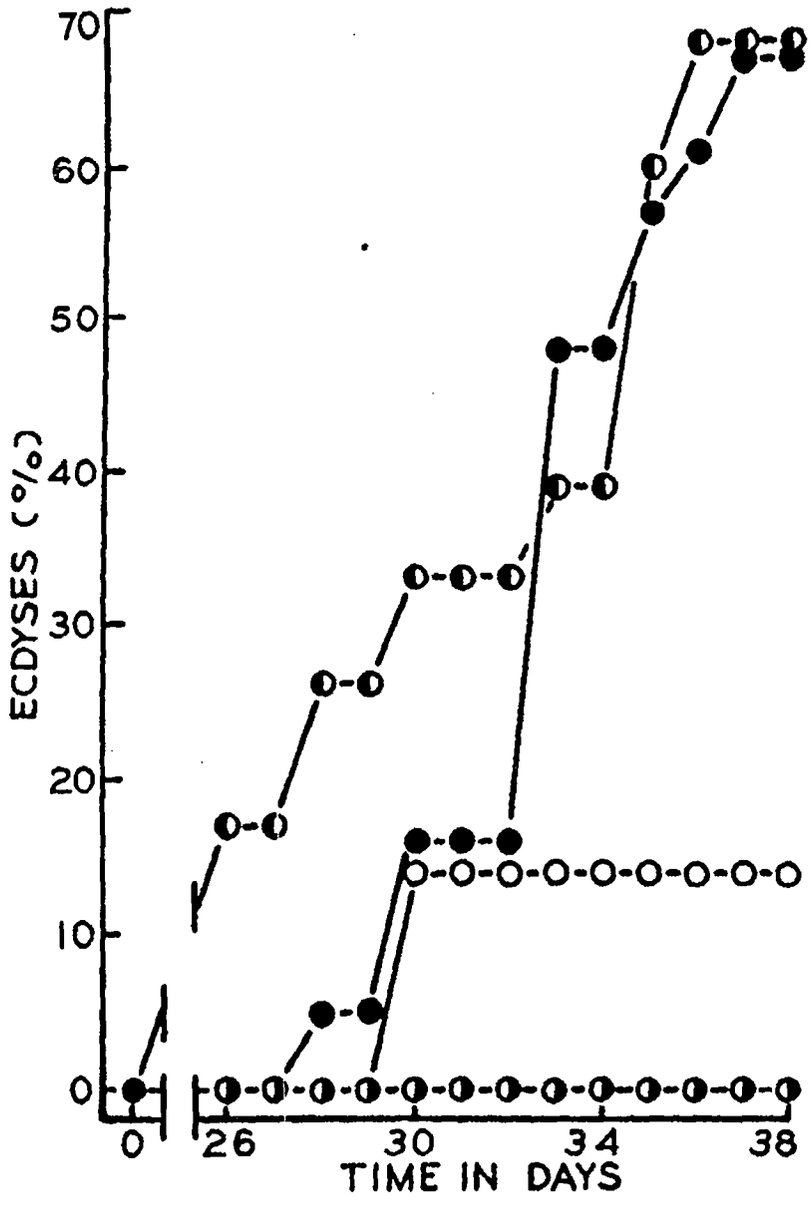
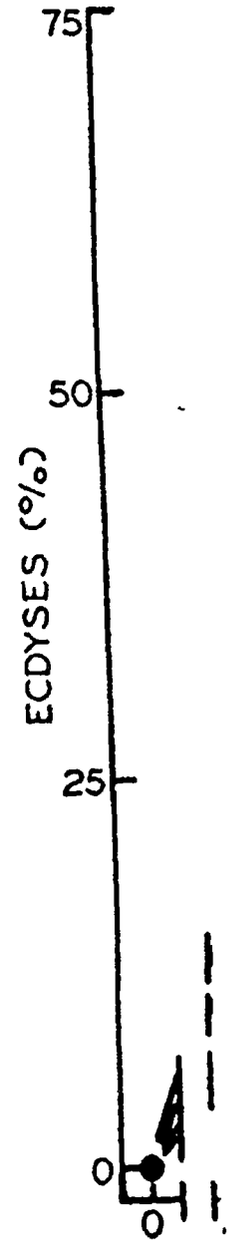


Figure 1. Effect of Aroclor 1242 on the rate of ecdysis of the fiddler crab, Uca pugilator. Empty circles, crabs lacking four walking legs in Aroclor 1242; filled circles, control crabs lacking four walking legs; circles half-filled on right, eyestalkless crabs in Aroclor 1242; circles half-filled on left, eyestalkless control crabs.



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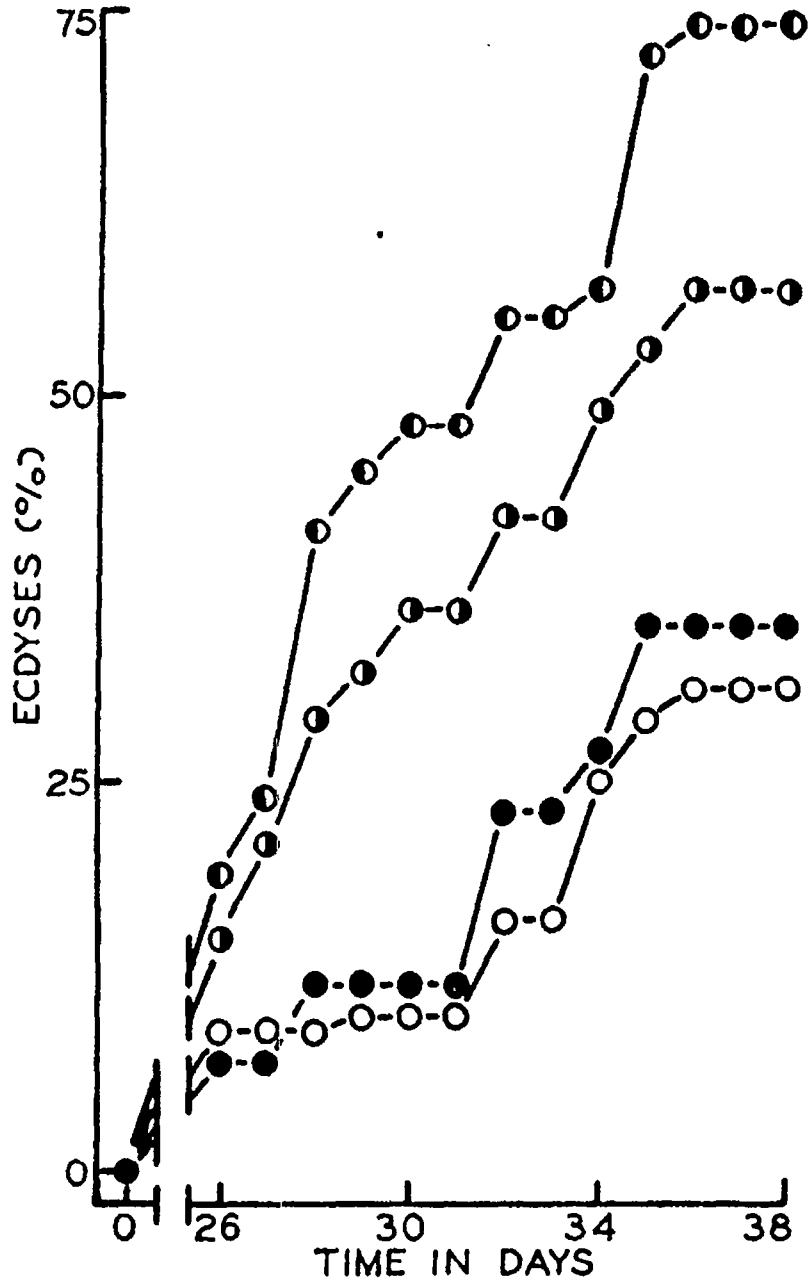

Figure 2. Effect of 1,2,3,4,5,6,7,8-octachlorodibenzofuran (OCDF) on the rate of ecdysis. Empty circles, crabs lacking four walking legs in OCDF; filled circles, control crabs lacking four walking legs; circles half-filled on right, eyestalkless crabs in OCDF; circles half-filled on left, eyestalkless control crabs.

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the incidence of ecdysis was determined (Figure 2). The two identical experiments of this set were started July 8, 1976. The control crabs lacking eyestalks or four walking legs underwent ecdysis at rates only slightly greater than those of the corresponding groups in the OCDF, the difference between the experimental and control groups being somewhat larger in the case of the eyestalkless crabs than with the crabs lacking four walking legs.

Discussion

Aroclor 1242 drastically inhibits the rate of molting of fiddler crabs lacking either both eyestalks or four walking legs (Figure 1). In fact, Aroclor 1242 completely inhibited ecdysis among the eyestalkless crabs. However, the OCDF produced only a relatively slight inhibition of the rate of molting (Figure 2). It is, therefore, highly likely that only a very small proportion of the inhibition caused by Aroclor 1242 would have been due to PCDF contaminants. Whereas by the end of the experiments with the Aroclor, the ratio of the percent ecdysis of the eyestalkless crabs in the Aroclor to that of the control crabs was 0.00 and the corresponding ratio for the crabs lacking four walking legs was 0.21, the ratios derived from the OCDF experiments were 0.77 for the eyestalkless crabs and 0.89 for the crabs lacking four walking legs. Therefore, although on an equal weight basis, PCDFs appear to be much more toxic to chicks than are PCBs (VOS et al., 1970), the vast proportion of the inhibition of molting activity in the fiddler crab produced by Aroclor 1242 appears due to the PCB itself and not to the small quantity of PCDFs that are contaminants in Aroclors.

Acknowledgement

This investigation was supported in part by USPHS Grant Number 5-SDS-RR07040-08.

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Vol. 18, No. 2 © 1977 by Spri

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