Toxicity of Polychlorinated Biphenyls (Aroclor 1254) to Adult, Juvenile, and Larval Stages of the Shrimp *Palaemonetes pugio*

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Polychlorinated biphenyls (PCBs) are recognized as widespread environmental contaminants (RISEBROUGH et al. 1968). The accidental release of Aroclor 1254 into a Florida estuary has been associated with "fish kills" and high residue levels in organisms and sediment (DUKE et al., 1970). Relatively few studies have considered PCB toxicity with respect to the early life stages of marine and estuarine animals. Juvenile pink shrimp Penaeus duorarum were more sensitive to Aroclor 1254 than were adults (NIMMO et al., 1971). A concentration of 0.94 ppb killed 51% of juveniles in their study in 15 days, while 3.5 ppb resulted in 50% mortality of adults in 35 days. HANSEN et al. (1971) found that 5 µg/liter Aroclor 1254 was lethal to juvenile Lagodon rhomboides and Leistomus xanthurus, estuarine fishes, in 14 to 45 days. With the sheepshead minnow Cyprinodon variegatus, hatching success was reduced at Aroclor 1254 levels above 10 µg/liter while survival of fry was inhibited above 0.1 µg/liter (SCHIMMEL et al., 1974).

We report in this paper the toxicity of Aroclor 1254 to adult, juvenile and larval stages of the grass shrimp, Palaemonetes pugio, an estuarine crustacean abundant along the Atlantic and Gulf of Mexico coastal regions of North America. Toxicity and uptake of Aroclor 1254 by adult P. pugio (NIMMO et al., 1974) as well as avoidance behavior of P. pugio to Aroclor 1254 (HANSEN et al., 1974) have been previously reported.

MATERIALS AND METHODS

Adult (3.0 to 4.0 cm total length) and juvenile (1.5 to 2.0 cm) grass shrimp Palaemonetes pugio were collected from Galveston Bay, Texas in the vicinity of Galveston Island. Larvae hatched from gravid females held in the laboratory. Seawater was prepared from Instant Ocean Synthetic Sea Salts. Studies were conducted at room temperature (19 to 21°C) and salinities of 15 o/oo S in the case of adults, 1 to 35 o/oo S with juveniles and 20 o/oo S with larvae. Salinity was measured with an AO Refractometer.

Aroclor 1254 (PCBs with 54% chlorine content) was obtained from the Monsanto Company. Analyses

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for Aroclor 1254 in exposure media were conducted according to the gas chromatographic method described by GIAM et al. (1972). Since it was determined that nominal concentrations did not accurately represent levels of Aroclor 1254 in exposure media, only measured concentrations are reported in this paper. Shrimp were exposed to seawater containing Aroclor 1254 dissolved in an acetone carrier.

All experiments were conducted under static conditions as described in A.P.H.A. et al. (1971). Glassware were rinsed with acetone prior to use.

Adult shrimp. Bioassays were conducted for 96 h in glass aquaria containing 5 liters seawater, 10 shrimp per aquarium. Aeration was provided by slowly bubbling charcoal-filtered compressed air through glass pipettes. Exposure concentrations of 0, 7.0, 28 and 72 µg/liter were prepared by pipetting the appropriate amounts of an Aroclor 1254 stock solution beneath the water surface and stirring. Water samples for PCB analysis were obtained prior to the addition of animals and at 24, 48, and 96h of exposure. Aquaria were examined daily for dead shrimp which were discarded.

Since we were concerned with the possibility of synergism between Aroclor 1254 and acetone, bioassays were conducted with a seawater-Aroclor 1254 mixture which was prepared without acetone and a similar mixture to which a known amount of acetone was added. To prepare the seawater-Aroclor 1254 mixture without acetone, seawater was "partially equilibrated" with Aroclor 1254. This was accomplished by adding 10 gm Aroclor 1254 to 15 liters of seawater in a Pyrex carboy and stirring for 3 days on a magnetic stir plate, followed by the addition of a blended mixture of one "drop" ($\sim$ 0.2 gm) Aroclor 1254 and 500 ml seawater to the carboy. The contents were stirred for an additional 11 days. This procedure was employed since true equilibration of Aroclor 1254 with water requires stirring for 2 months (HAQUE et al.,1973). Our "partially equilibrated" seawater had an Aroclor 1254 concentration of 150 µg/liter. Lower concentrations of Aroclor 1254 were obtained by adding 15 o/oo S seawater to the "partially equilibrated" solution. One ml acetone was pipetted directly into exposure aquaria containing 5 liters of the mixture described above, in tests for the combined effects of Aroclor 1254 and acetone. The nominal acetone concentration, 200 mg/liter, represents the maximum level used in this study, and was not toxic to shrimp.

Juvenile shrimp. Experimental conditions for juvenile shrimp were essentially similar to those described for adults. Bioassays were conducted at 1, 7, 14, 21, 28 and 35 o/oo S. One week was allowed for salinity acclimation prior to bioassays. Shrimp were exposed to concentrations of 0, 1.4, 16 and 70 µg/liter at each salinity.

Larval shrimp. One day old larvae were transferred with a wide-bore pipette to 4 inch fingerbowls with 150 ml of seawater containing Aroclor 1254, 10 larvae per bowl. Bowls were examined daily for dead individuals which were discarded. Every second day, larvae were transferred to clean fingerbowls containing freshly prepared exposure media and fed newly hatched Artemia nauplii (San Francisco). Larvae were reared to postlarvae in this manner.

Larvae obtained from 3 gravid shrimp were used in this experiment. Each hatch consisted of approximately 50 larvae. Forty larvae from each hatch were distributed equally among the exposure concentrations of 0, <0.1, 3.2 and 15.6 µg/liter Aroclor 1254. The <0.1 µg/liter concentration represents levels of Aroclor 1254 below the detection limit of our analytical procedure.

RESULTS

Concentrations of Aroclor 1254 in exposure media decreased with time according to the loss curve shown in Figure 1. Since there was no consistent relationship between initial exposure concentration and loss rate, values in Figure 1 were expressed as the percentage of the initial concentration. The decrease of Aroclor 1254 can probably be attributed to volatilization and, to a lesser extent, by uptake of organisms and adsorption to the glass walls of exposure containers. Such phenomena create difficulties in toxicity analysis since organisms are not exposed to a constant level of toxicant throughout the experiment. We have elected to use the initial exposure concentration in expressing the toxicity of Aroclor 1254 to Palaemonetes pugio. Although such a practice does not completely describe our experimental situation, relative toxicities to organisms under similar conditions would not be altered. In the larval experiment, concentrations fluctuated in 2-day cycles since exposure media were renewed every second day.

Ninety-six hour LC-50 values for adult shrimp are presented in Table 1A. Since the presence of 200 mg/liter acetone did not decrease the 96 h LC-50 of the "partially equilibrated"

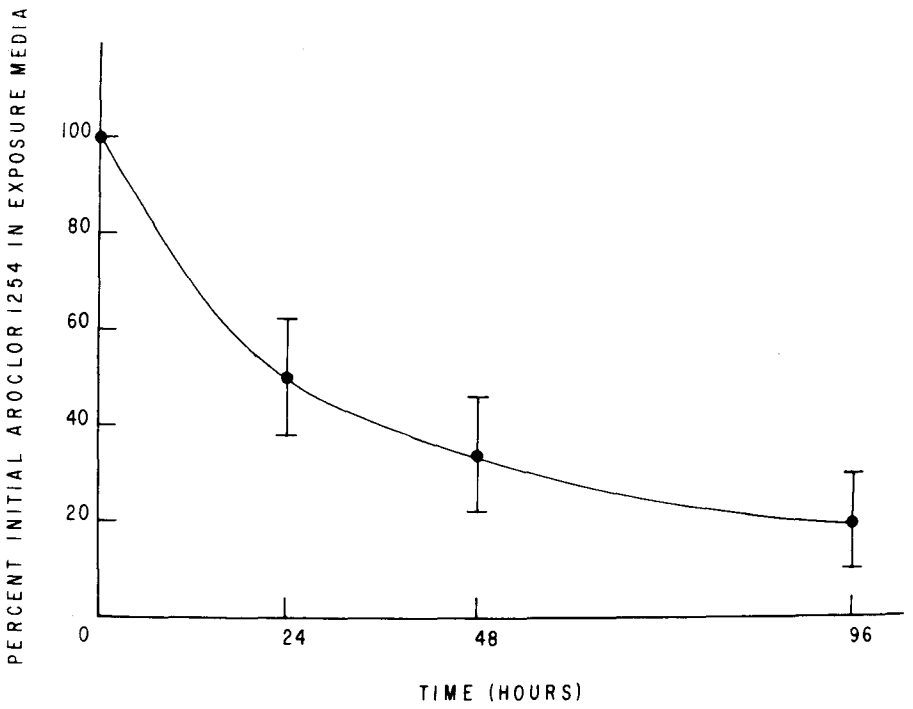

Figure 1. Relative decrease of Aroclor 1254 in exposure media. Points on the curve are means of percentages of initial concentrations remaining at the times indicated. Vertical bars indicate 95% confidence intervals of means. Angular transformation was applied to per cent values to calculate means and 95% C.I.'s.

TABLE 1

Toxicity of Aroclor 1254 to adult and juvenile Palaemonetes pugio: 96 h LC-50 values.

A. Adults

Test Solution	96 h LC-50 (µg/liter)
Aroclor 1254 equilibrated directly with 15 o/oo seawater.	64
Aroclor 1254 equilibrated with seawater (5 liters) plus 1 ml acetone (= 200 mg/liter acetone)	86
Aroclor 1254 dissolved in acetone	41

B. Juveniles

Salinity (o/oo S)	96 h LC-50 (µg/liter)
1	6.1
7	6.1
14	7.8
21	7.8
28	7.8
35	7.8

seawater, it was concluded that synergism by acetone and Aroclor 1254 did not occur in our study. The slightly lower 96 h LC-50 of 41 µg/liter for shrimp exposed to Aroclor 1254 dissolved in acetone may have resulted from the PCBs occurring in a more finely dispersed state due to the activity of the solvent carrier.

Juvenile shrimp with 96 h LC-50 values from 6.1 to 7.8 µg/liter (Table 1B) were more sensitive than adults. Values were slightly lower at the lower salinities, but the magnitude of the difference was so small that any effect of salinity on Aroclor 1254 toxicity was not apparent.

Development of P. pugio to postlarvae occurred at exposure concentrations below 15.6 µg/liter (Table 2). At 15.6 µg/liter, 75% of the larvae died on the fifth and sixth days

TABLE 2

Toxicity of Aroclor 1254 to larval Palaemonetes pugio: survival and duration of larval development.

Aroclor 1254 concentration (µg/liter)	Number of larvae	Percent survival	Days to postlarvae
0 (acetone control)	30	93	22.7 ± 1.6
<0.1	30	100	24.0 ± 2.6
3.2	30	90	26.4 ± 3.8
15.6	30	0	--a

--a. 75% died on the 5th and 6th days of exposure at 15.6 µg/liter. No larvae survived longer than day 11.

of exposure and all died within 11 days. At concentrations of 3.2 and <0.1 µg/liter, percent survival to the postlarval stage did not differ from that of controls. However, the duration of development to postlarvae increased as the concentration increased from 0 to 3.2 µg/liter. Controls averaged 22.7 days to postlarvae. Mean larval duration for groups exposed to <0.1 µg/liter was 24.0 days, and those exposed to 3.2 µg/liter was 26.4 days. Differences among means were statistically significant at $p < 0.10$ (Table 3). Although this level of significance is relatively high, the observed differences may be of biological consequence.

TABLE 3

Analysis of variance of days to postlarvae of Palaemonetes pugio larvae reared in different concentrations of Aroclor 1254.

Source of variation	Degrees of freedom	Sums of squares	Mean square	F_s
Concentration of Aroclor 1254	2	189	94.5	2.85[a]
Error	82	2714	33.1	
Total	84	2903		

[a]Significant at $p < 0.1$; $F_{2, 60; \alpha = 0.1} = 2.39$

Discussion

Larval and juvenile Palaemonetes pugio were more sensitive to Aroclor 1254 than adults. This was expected since earlier life stages are generally considered to be more sensitive to the physicochemical nature of the environment (THORSON, 1957). Larvae were able to develop to postlarvae at 3.2 µg/liter, a concentration equivalent to approximately half the 96 h LC-50 of juvenile shrimp. Differences in salinity did not appreciably alter the toxicity of Aroclor 1254 to juvenile grass shrimp. Our toxicity data for adult P. pugio (96 hr LC-50 approximately 60 µg/liter) are not directly comparable to those reported by NIMMO et al. (1974) since the latter study utilized a continuous-flow exposure system and reported toxicity for longer exposure periods (9 and 16 days). Shrimp in their study appeared to exhibit a greater sensitivity to Aroclor 1254. As demonstrated by Nebeker and Puglisi (1974), PCBs are more toxic under continuous-flow conditions than static, due to the continuous renewal of toxicant.

The effect of Aroclor 1254 on P. pugio larvae was evident both as mortalities at a relatively high concentration of 15.6 µg/liter and increased duration to the postlarval stage at lower exposure levels. Larval grass shrimp are not as motile as postlarvae, and extended larval development may be associated with greater predation or more difficult food capture. Changes in the duration of larval development of P. pugio have also been observed to occur in response to differing diets (BROAD, 1957).

NEBEKER and PUGLISI (1974), NEBEKER et al. (1974) and SCHIMMEL et al. (1974) have shown that low concentrations of PCBs not lethal to adult organisms within a specified period are detrimental to normal development in several aquatic arthropods and fish. The observed effects have included reduced hatching success, increased mortality of early stages, and, in the case of the midge Tanytarsus dissimilis (NEBEKER and PUGLISI, 1974), inhibition of metamorphosis. Increased abnormalities in chick embryos, as well as reduced hatching, have also been observed when eggs were injected with Aroclor 1242 (MCLAUGHLIN, 1963) and when hens were fed various PCBs prior to egglaying (CECIL et al., 1974).

Our results and the studies discussed above suggest a teratogenic action by PCBs. It appears that increased susceptibility of early stages of organisms to PCBs may be a general phenomenon.

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