Exhibit # 6

ACCUMULATION OF AROCLOR® 1254 IN GRASS SHRIMP (Palaemonetes pugio) IN LABORATORY AND FIELD EXPOSURES

by

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Results of several experiments indicate that aquatic invertebrates accumulate total body concentrations of polychlorinated biphenyls (PCB) thousands of times greater than that of the surrounding water. For example, Sanders and Chandler (1) showed that fresh water insects and crustaceans rapidly (1 day) accumulated PCB (Aroclor 1254) up to 24,000 times greater than the concentration in the water. Results of similar exposures conducted with estuarine animals showed oysters concentrating 85,000 (2), shrimp 10,000 (3) and fish 30,000 (4) times the amount of PCB in the water.

Although Sanders and Chandler stated that PCBs entering the aquatic environment are below concentrations acutely toxic to invertebrates (1), we have noted that most of the accumulation studies conducted thus far by the investigators cited in the paragraph above have been at concentrations of 1.0 µg/ℓ and above, i.e., concentrations demonstratively toxic to test animals. Little is known about accumulation in marine invertebrates at extremely low concentrations, and with one exception (3), no one to our knowledge has placed PCB-free animals in a natural environment known to have PCBs and followed accumulation with time.

We report here the results of several experiments on chronic toxicity of Aroclor 1254 to Palaemonetes pugio, an estuarine grass shrimp, as well as concentration and loss of the compound from the animals with time. We also exposed grass shrimp for up to 3 months to Aroclor 1254-contaminated sediments in Escambia Bay, near Pensacola, Florida.

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METHODS AND MATERIALS

With one exception, all laboratory experiments were conducted in 30-ml chambers supplied with flowing water from Santa Rosa Sound. Three sets of 5 chambers each received test concentrations; the fourth set was a control. Each chamber contained 4-10 shrimp, the number depending on the size of the animals. Water flowed continuously through each chamber at a rate of 1.0 l/hr. Aroclor 1254, dissolved in polyethylene glycol (mol. wt. 200), was metered into each mixing tank with a syringe pump before the water entered the test chamber. An equal amount of solvent was added to the water flowing to controls. David J. Hansen of this laboratory, found the sensitivity of a marina fish to Aroclor 1254 remained unchanged when he varied concentrations of polyethylene glycol used to deliver the toxicant (personal communication). The shrimp were fed daily a commercial molly-flake diet (<0.02 mg/kg organochlorine compounds).

The experiment to determine the concentration and loss from the tissues of P. pugio was also conducted in a flowing-water system. We constructed 18-liter aquaria with false floors of nylon screen (1/4-inch mesh) to hold shrimp above the detritus brought in with the water or produced by the animals. This modification was intended to prevent the animals from eating these particles with adsorbed Aroclor 1254. Consequently, we assume that shrimp obtained more of the chemical from the water by absorption through the gills rather than from ingestion of contaminated detritus. The shrimp were not fed during this experiment.

Concentrations of Aroclor 1254 in tissues by gas chromatography were determined using pooled samples of at least 10 shrimp each (5).

P. pugio were exposed to Aroclor 1254-contaminated sediments in upper Escambia Bay from November 1971 to February 1972. The shrimp in specially-constructed cages (5) were exposed directly to the sediments. Average concentration of Aroclor 1254 in the uppermost two inches of sediment in November 1971 was 5.0 mg/kg (dry weight),

RESULTS OF LABORATORY EXPOSURES

Tests conducted in flowing water showed P. pugio to be susceptible to Aroclor 1254 (Table 1). In a 7-day exposure, 60% died at 9.1 µg/l, but significant mortality did not occur at 0.17 and 0.62 µg/l. In the second series of tests lasting 16 days, 4.0 and 12.5 µg/l were toxic, but significant mortality did not

TABLE 1.

MORTALITY AND ACCUMULATION OF AROCLOR 1254 IN *Palaemonetes pugio**

Test Conc. (µg/ℓ)	Days Exposed	Average Mortality (%)**	Body Conc. (mg/kg)	Concentration Factor
CONTROL	7	4(0 - 20)	0.1	———
0.17	7	8(0 - 40)	1.3	7600
0.62	7	4(0 - 20)	5.4	8700
9.1	7	60(20 - 80)***	65.0	7100
CONTROL	16	25(0 - 50)	<0.1	———
1.3	16	40(0 - 100)	18.0	14000
4.0	16	45(25 - 50)***	27.0	6700
12.5	16	55(50 - 75)***	46.0	3700

*All exposures were conducted in flowing seawater: salinity and temperature ranges were 22 to 28‰ and 17 to 28° C.

**5 replicates per concentration: at least 4 shrimp per replication.

***Significant at P >0.05.

TABLE 2.

ACCUMULATION OF AROCLOR 1254 BY
*Palaemonetes pugio**

Test Conc. (µg/ℓ)	Body Conc. (mg/kg)	Concentration Factor
0.17	1.3	7600
0.62	5.4	8700
1.0	3.2	3200
2.3	25.0	11000
2.7	19.0	7000
3.2	15.0	4800
3.2	26.0	8100
5.2	29.0	5600
5.3	16.0	3000
5.3	30.0	5700
9.1	65.0	7100

*7-day exposures conducted in flowing seawater at salinity and temperature ranges of 22 to 28 and 17 to 28° C.

occur in 1.3 µg/ℓ.

At the conclusion of several one-week exposures to a range of concentrations (0.17 to 9.1 µg/ℓ), surviving shrimp from each exposure were analyzed for whole-body residues. Ambient concentration of toxicant in the water and resultant residues in the shrimp were correlated (r=0.91, Table 2). In some cases, duplicate test concentrations produced biological accumulations that differed by a factor of 2. Concentration factors ranged from 3,000 to 11,000. These ranges were similar to those found in tests using penaeid shrimp (3) but were somewhat lower than those found by Sanders and Chandler (1), in tests using several invertebrate species in fresh water.

There appeared to be no threshold below which levels of the chemical added to water failed to produce residues in the tissues (Table 3) in our tests. Whole-body concentrations produced after 5 weeks exposure to 0.04, 0.09 and 0.62 µg/ℓ ranged from 200 to 26,000 times the concentrations in the test water. Concentrations did not reach equilibrium and from 60 to 90 percent of the Aroclor 1254 was lost from the shrimp within 4 weeks after exposure to the chemical was stopped. Test concentrations of the chemical were not significantly toxic to shrimp. Although accumulation increased with increasing concentration of toxicant in this test, this was not observed in earlier studies (see Tables 1 and 2). Implications are to be discussed elsewhere.

RESULTS OF FIELD EXPOSURES

Average whole-body residue of Aroclor 1254 in P. pugio after 1 month was 0.41 mg/kg (0.34 to 0.57); after 3 months, 0.42 mg/kg (0.37 to 0.50). There was no evidence that significant mortality occurred during the exposures of grass shrimp to contaminated sediments.

DISCUSSION

Concentrations of Aroclor 1254 [illegible] exposure to contaminated sediments [illegible] equivalent to a laboratory exposure of 0.09 µg/ℓ for 2 weeks (Table 3). We expected residues to be higher in caged shrimp since we had found that fiddler crabs exposed in the laboratory accumulated residues equal to or greater than (wet-weight basis) that of the contaminated substratum (dry-weight basis) after 30 days (6). Concentrations of Aroclor 1254 in caged shrimp exposed to contaminated sediments appeared to reach a plateau, but this was not the case in laboratory exposures (Table 3) where an equilibrium

TABLE 3.

ACCUMULATION OF AROCLOR 1254 IN *Palaemonetes pugio* WITH TIME AFTER EXPOSURES TO THE CHEMICAL IN WATER AT THREE CONCENTRATIONS (µg/ℓ)
(Each value represents a composite sample of 10 animals)

Length of Exposure (hr/ days)	Control		0.04		0.09		0.62	
	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor
0	0.1	*	0.1	*	0.1	*	0.1	*
1	—	*	0.1	*	0.1	*	0.1	*
2	—	*	0.1	*	0.1	*	0.1	*
3	—	*	0.1	*	0.1	*	0.1	*
4	—	*	0.1	*	0.1	*	0.1	*
8	—	*	0.1	*	0.1	*	0.12	190
12	—	*	0.1	*	0.1	*	0.14	230
16	—	*	0.1	*	0.1	*	0.26	420
24 / 1	—	*	0.1	*	0.1	*	0.20	320
36 / 1.5	—	*	0.1	*	0.1	*	0.20	470
48 / 2	—	*	0.1	*	0.10	1100	0.37	600
72 / 3	—	*	0.1	*	0.14	1560	0.58	930
96 / 4	—	*	0.1	*	0.15	1670	0.40	650
154 / 6.5	0.1	*	0.1	1590	0.33	3670	1.28	2060
336 / 14	—	*	0.13	3250	0.43	4780	7.40	11930
504 / 21	0.1	*	0.15	3750	0.45	5000	6.67	10900
672 / 28	0.14	*	0.17	4250	1.57	17400	10.82	17450
840 / 35	0.10	*	0.21	5250	0.75	8330	16.48	26580
			PCB - STOPPED					

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TABLE 3 (Continued)

1176 / 49	0.1	*	0.1	*	0.12	*	3.24	*
1512 / 63	0.15	*	0.1	*	0.13	*	1.64	*

* Magnification factor not calculated.

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was not reached. Therefore, we believe that shrimp exposed to the sediments might have obtained PCB from the water or food singly, but shrimp exposed to Aroclor in the laboratory obtained chemical from two sources, water and food. It might also be more available in the laboratory than the field due to the carrier. In earlier laboratory studies with penaeid shrimp, both water and food appeared to be sources (3).

No significant mortality was observed in caged shrimp and none would be predicted since residues produced in the field were similar to those found in shrimp after laboratory exposures which caused no death.

Penaeid shrimp spend only a fraction of their life cycle in an estuary, moving into oceanic waters after reaching maturity (7), but grass shrimp are endemic in estuaries. Therefore, in relation to time of exposure we would expect grass shrimp to accumulate a pollutant from a contaminated estuary to a greater degree than penaeid shrimps, nevertheless, this is not true. In August 1968, penaeid shrimps (Penaeus duorarum, P. setiferus, and P. aztecus) collected during a survey of Escambia Bay, Florida, had whole-body residues of Aroclor 1254 as high as 14.0 mg/kg (3). In that survey and in subsequent collections, P. pugio had a maximum residue of only 1.4 mg/kg.

Lower residues in P. pugio from Escambia Bay may be due to amounts of PCB in bay sediments and behavioral patterns of the animals. We noted earlier (3) that residues in species of penaeid shrimp were related to higher concentrations of Aroclor 1254 in sediments that predominate in upper Escambia Bay. We found that penaeid shrimp, as adults, usually are captured in deeper waters and burrow into silty or sandy substrates. In contrast, grass shrimp usually do not burrow, rather are found along shallow sandy beaches and grass beds, where they obtain food that is relatively uncontaminated with PCB.

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