A Polychlorinated Biphenyl (Aroclor 1254®) in the Water, Sediment, and Biota of Escambia Bay, Florida[1]

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We have detected a polychlorinated biphenyl (PCB), Aroclor 1254, in the biota, sediment, and water of estuarine areas near Pensacola, Florida. Only one source of the chemical, an industrial plant on the Escambia River, has been located. However, the chemical occurs in tissues of pelagic and sessile organisms that are widely distributed within the estuary. This distribution of Aroclor 1254 could be due to dispersion of the chemical from the river by currents and biota to other parts of the system. Alternatively, it could have entered the system from more than one source. In this paper we report the occurrence of Aroclor 1254 in the estuarine environment and discuss its possible effects on some estuarine organisms.

PCB's are considered industrial pollutants and, unfortunately, are relatively persistent in an aquatic ecosystem. Aroclor 1254, one of a series of chlorinated biphenyl compounds manufactured by the Monsanto Company, is a light yellow viscous oil with a chlorine content of 54%. They are used in industry as plasticizers and resins for chlorinated-rubber-based lacquers, varnishes and paints, lubricants, heat transfer fluids and insulators. Also, PCB's are used as carriers for some insecticides. They are stable, insoluble in water and highly soluble in lipid. Structurally they resemble chlorinated hydrocarbon insecticides such as DDT. Thus, it is not surprising to find PCB's sorbed onto sediment and accumulated in the tissues of animals exposed to water containing this chemical.

Polychlorinated biphenyls occur in fish and wildlife from other parts of the United States, Great Britain and the Netherlands. Risebrough (1) reported these compounds to be widely distributed in marine ecosystems of the Pacific Ocean. Marine birds had higher concentrations of PCB's than fish, and animals from San

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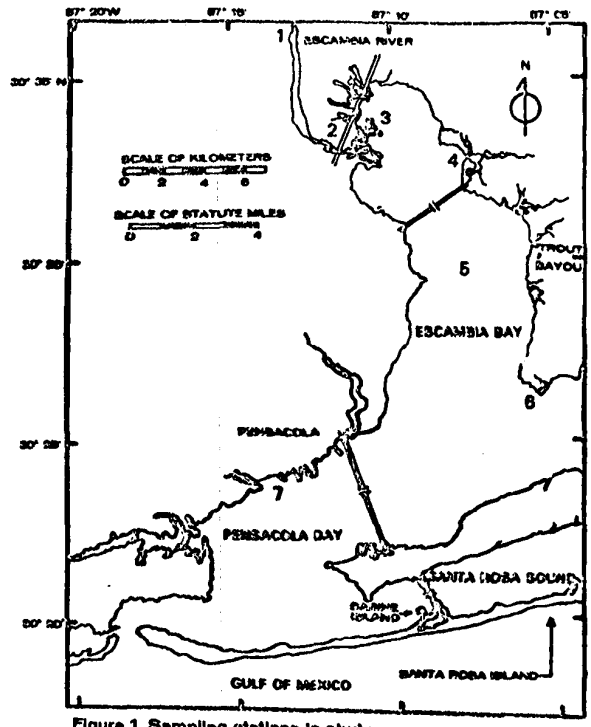

Figure 1. Sampling stations in study area.

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Francisco Bay contained more of the chemicals than those from the ocean. A recent survey by this laboratory of the pesticide content of estuarine animals from Charleston, South Carolina, revealed less than 0.1 ppm (mg/Kg) of PCB in fish and blue crabs. Also, fish and shellfish from Texas and Georgia, collected as part of the Bureau of Commercial Fisheries National Monitoring Program, contained residues of these chemicals, as did coastal birds and bird eggs from England (2) and mussels, fish, and birds from The Netherlands (3).

Field Studies

We first detected residues of Aroclor 1254 in oysters in April 1969 at a newly established pesticide monitoring station in Escambia Bay. Subsequent sampling in this area showed residues of the chemical in water, sediment, fish, blue crabs, and shrimp. We traced one source of Aroclor 1254 to the outfall of a local industry (located 6 miles upstream from Station 1, Figure 1) after analyzing 30 water samples from Escambia River and Bay. The Aroclor reportedly entered the plant's effluent through accidental leakage of a heat-exchange fluid.

Methods. Samples of biota, water, and sediments were collected from April through October, 1969. Some of the animals were dead or moribund when collected. They were taken from the water immediately after "fish-kills" that were reportedly due to lack of oxygen in the water. Others were captured alive by trawl. Water was collected in 1-gallon glass jugs. Sediment was taken with a modified grab sampler.

The tissues of fish, crabs, oysters, and shrimp were mixed with anhydrous sodium sulfate in a blender. The mixture was extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and partitioned with acetonitrile. The acetonitrile was evaporated just to dryness and the residue transferred to a Florisil column (4) with petroleum ether. Aroclor 1254 was eluted from the column with 6% ethyl ether-in-petroleum ether.

Sediment samples were dried at room temperature and extracted for 4 hours with 10% acetone in petroleum ether in a Soxhlet apparatus. Extracts were evaporated just to dryness and the residues eluted from a Florisil column.

Water samples were extracted with petroleum ether. The extracts were dried with sodium sulfate and reduced to an appropriate volume.

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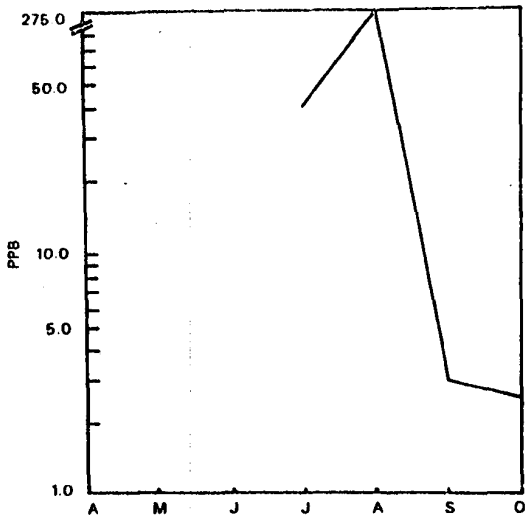

Figure 2A. Aroclor 1254 in water from outfall in Escambia River.

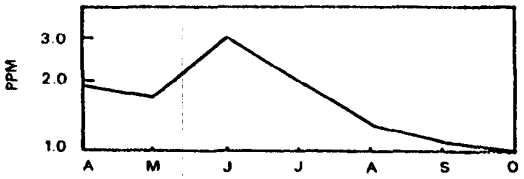

Figure 2B. Aroclor 1254 in live oysters from Escambia Bay (Station 5).

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The extracts of all substrates were identified and measured by electron capture gas chromatography. Three columns of different polarity (DC-200, QF-1, mixed DC-200 and QF-1) were used to confirm identification. In a few samples, thin layer chromatography was employed for additional confirmation and to assess the amount of DDT present. Interference from DDT was negligible, due to the relatively high residues of Aroclor 1254 found in the samples. Quantitation of this multiple peak compound was approximated by averaging the peak height of five major peaks. Laboratory tests indicated recovery rates above 80%. Data in this report do not include a correction factor for percentage recovery.

Residues of Aroclor 1254 in biota, water, and sediment of Escambia Bay. Evidently, Aroclor 1254 moved from the water of Escambia River to biota and sediment in the Bay and contiguous waters (Table 1). Oysters are excellent indicators of the presence of the Aroclor in the environment and reflect, in general, the amount of the chemical in the water (Figure 2B). Fish, shrimp, and crabs contained higher concentrations of the chemical than oysters but because of their mobility they are not as useful as monitors for a particular area. Highest concentrations of Aroclor in water during the sampling period occurred at the outfall in Escambia River in August and decreased abruptly when leakage from the plant was corrected (Figure 2A). Less than 0.1 ppb (µg/l.) of the Aroclor occurred in the water at Station 2 (Figure 1) but it was not detected in lower bay water. Continued presence of the chemical in river water presumably is a result of leaching from sediments. Aroclor residues in sediment samples taken near the outfall reached 486 ppm in August but have decreased since that time.

Laboratory Studies

The presence of PCB's in terrestrial and aquatic animal tissues is well documented, but to our knowledge, there are no published data on the toxicity of these compounds to marine life. In order to evaluate the concentrations of Aroclor found in water and animal tissues in relation to toxicity, we conducted bioassays under controlled conditions in the laboratory. Fish, shrimp, and oysters were exposed to Aroclor 1254 in acute (96 hours or less) laboratory bioassays. Also, juvenile pink shrimp, Penaeus duorarum, and blue crabs, Callinectes sapidus, were exposed to concentrations of Aroclor 1254 for 20 days. The animals were analyzed for residues of this PCB.

Bioassay procedures. The acute toxicity of Aroclor 1254 to marine animals was determined by exposing separate populations of juvenile fish, shrimp, and oysters to three concentrations (100.0,

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TABLE I

Residues of Aroclor 1254 in samples from Escambia Bay and River

Sample (shrimp collected live; crab and fish were dead or moribund)*	Number of animals or organs in sample	Date collected (D-M-Y)	Location (See Fig. 1)	Residues of Aroclor 1254 ppm
Fish				
Flounder liver	1	5-6-69	Mulatto Bayou (4)	76.
Flounder muscle	1	"	"	4.5
Flounder gills	1	"	"	19.
Croaker	3	"	"	12.
Menhaden	5	"	"	11.
Pinfish	5	"	"	10.
Menhaden	5	1-7-69	"	12.
Speckled trout gills	1	14-8-69	Pensacola Bay (7)	7.5
Speckled trout liver	1	"	"	21.
Speckled trout	3	31-8-69	Northern Escambia Bay (3)	20.
Flounder liver	1	"	"	184.
Menhaden	6	10-10-69	Mulatto Bayou (4)	5.7
Crustaceans				
Shrimp	10	1-7-69	Mulatto Bayou (4)	1.5
Shrimp	5	31-8-69	Northern Escambia Bay (3)	2.5
Blue crab	6	13-8-69	"	7.0
Blue crab	5	31-8-69	"	6.3
Blue crab	6	9-9-69	Mulatto Bayou (4)	1.0
Sediment				
"		8-7-69	Highway 90 (2)	1.7
"		15-8-69	Garcon Point (6)	<.03
"		"	Outfall 6 miles north of (1)	486.

* Scientific names: trout (Cynoscion nebulosus), croaker (Micropogon undulatus), menhaden (Brevoortia patronus), pinfish (Lagodon rhomboides), flounder (Paralichthys sp.), shrimp (Penaeus setiferus) and (Penaeus aztecus), and blue crab (Callinectes sapidus).

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10.0, and 1.0 ppb) of this PCB for 48 hours (96 hours for the oysters). The bioassay techniques used are those routinely employed at this laboratory to study the relative toxicity of pesticides to marine life. The animals are held in aquaria with flowing sea water to which acetone stock solutions of the pollutant are added (with stopcocks or metering pumps) to give the desired test concentration (5,6).

Bioassay animals were captured in local waters and acclimated to laboratory conditions. Ten pinfish, Lagodon rhomboides (1" - 1 1/2" long), 10 pink shrimp, Penaeus duorarum (2" - 3"), and 12 oysters, Crassostrea virginica (1" - 1 1/2") were exposed in separate aquaria to each concentration. Frequent observations were made on the fish and shrimp and percentage mortality recorded at the end of 24- and 48-hour periods. Rate of shell growth of each experimental oyster was measured at the end of 96 hours and percentage decrease (as compared to controls) calculated. The valve edges of the young oysters were ground smooth before the test. We have found that the rate of shell deposition in young, fast growing oysters is a convenient and objective method for evaluating effects of chemical pollutants on this mollusk (7).

The 20-day bioassays with juvenile crabs and shrimp were made under conditions similar to those used in the acute bioassays except the animals were confined in compartmented aquaria and acetone stock solutions of the Aroclor were added to the water with a dual channel metering pump. The desired concentration of Aroclor was 5.0 ppb in both experiments, and was selected on the basis of residues found in water samples from the lower reaches of Escambia River. Water samples taken from experimental aquaria at periodic intervals during the 20-day tests ranged from 3.5 to 4.2 ppb of Aroclor 1254. Control aquaria received the same amount of acetone (1 ml/min) as the experimental. Four hundred liters of sea water per hour flowed through each aquarium.

Shrimp were exposed from July 24 to August 13, 1969 and crabs from August 21 to September 10. Mean water temperature and salinity were 27°C and 28‰ during the first period and 28°C and 27‰ during the latter period.

Twenty-five juvenile pink shrimp (1 1/2" - 2 1/2") were used in each of two (control and experimental) compartmented aquaria and 20 blue crabs (1/2" - 2") in each of two aquaria. Individual shrimp and crabs must be isolated during a long-term experiment because of cannibalism. They are especially vulnerable to predation immediately after molting. All animals were fed pieces of fish at least twice a week.

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Results of acute bioassays. Tables II, III, and IV summarize the results of acute toxicity tests and the amount of Aroclor 1254 in the animals. This PCB appeared to have no effect on juvenile pinfish exposed to 100 ppb of the chemical for 48 hours. Residue analysis of these fish (whole body) revealed 17 ppm of the compound. As indicated in the tables, shrimp are susceptible to relatively low levels of Aroclor 1254. Shell growth of oysters exposed to 100 ppb of the PCB for 96 hours was completely inhibited. However, the oysters did survive and their shell growth equaled that of controls after 3 weeks in water that contained no detectable PCB's. Exposure at 100 and 10 ppb were repeated with almost identical results to those shown in Table IV. All animals concentrated the PCB.

Results of 20-day bioassays. Seventy-two percent (18 of 25) of the juvenile shrimp exposed to 5.0 ppb Aroclor 1254 died during the 20-day exposure. The first shrimp died on the tenth day of exposure, and a few died each day during the next 10 days. A composite sample of these shrimp contained 16 ppm of the PCB. The experiment was terminated after 20 days in order to have live shrimp for residue analysis. A composite sample of the seven surviving shrimp contained 33 ppm of the PCB. The shrimp did not exhibit typical symptoms of insecticide poisoning before death; that is, extreme irritability followed by loss of equilibrium. Several of the shrimp died immediately after molting. None of 25 control shrimp died during the 20-day experiment. Residue analysis of a composite sample of seven control shrimp revealed no PCB.

Juvenile blue crabs were not as sensitive as shrimp to Aroclor 1254. Only one of 20 crabs died during the 20-day exposure to 5.0 ppb. One control crab also died. The most striking aspect of this experiment was the relatively large amount of PCB concentrated in tissues of live crabs during the 20-day exposure and the high levels of PCB still present after the crabs were held in clean flowing sea water for 4 weeks. Five crabs removed for analysis after exposure contained an average whole body residue of 23 ppm Aroclor 1254 (range 18 to 27 ppm). Residues in five crabs held in clean water for 1 week averaged 22 ppm (range 10 to 37 ppm). Six crabs held in clean water for 4 weeks, following the 20-day exposure, contained an average of 11 ppm (range 3.0 to 14 ppm), an example of the persistence of this compound in animal tissue.

Implications of Study

From our data, we have gained insight into the movement of Aroclor 1254 in an estuary and its accumulation by the biota. Our labora-

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TABLE II

Acute toxicity of Aroclor 1254 to juvenile pinfish[1]

Test conc. ppb (µg/l)	Number of animals per concentration	Mortality - % 24 hrs	Mortality - % 48 hrs	Residues after 48 hrs exposure ppm (mg/kg)
100.0	10	0	0	17.00
10.0	10	0	0	3.80
1.0	10	0	0	0.98

[1] Mean temperature and salinity of sea water 16°C and 26‰.

TABLE III

Acute toxicity of Aroclor 1254 to juvenile pink shrimp[1]

Test conc. ppb (ug/l)	Number of animals per concentration	Mortality - % 24 hrs	Mortality - % 48 hrs	Residues after 48 hrs exposure ppm (mg/kg)
100.0	10	80	100	3.90
10.0	10	0	0	1.30
1.0	10	0	0	0.14

[1] Mean temperature and salinity of sea water 16°C and 26‰.

TABLE IV

Acute toxicity of Aroclor 1254 to oysters[1]

Test conc. ppb (µg/l)	Number of animals per concentration	Decrease in rate of shell growth in 96 hrs %	Residues after 96 hrs exposure ppm (mg/kg)
100.0	12	100	--
10.0	12	41	33.0*
1.0	12	19	8.1*

[1] Mean temperature and salinity of sea water 19°C and 31‰.

* Residues after 4 days in water that contained no detectable PCB's.

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tory studies showed that juvenile shrimp were the most sensitive, and were killed when exposed to 5.0 ppb of Aroclor 1254 in flowing sea water. The Aroclor content in water from Escambia Bay, even near the mouth of the river, contained less than 1 ppb. Shrimp collected from the bay contained a maximum of 2.5 ppm. Thus, the shrimp in Escambia Bay probably were not exposed to lethal levels of the chemical during the sampling period.

This study demonstrates the urgent need for continued surveillance of our estuaries in order to preserve those nursery grounds for our valuable fishery resources. Also, the study shows a need for conducting long-term tests on the effect of sublethal concentrations of Aroclor 1254 on estuarine organisms in sensitive stages of their life history.

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