E. P. Wheeler

1) Face
2) Paul
3) Gene
4) Boom cane

TOXICITY OF [redacted] AND ITS PHYSIOLOGICAL ACTIVITY IN SEVERAL ESTUARINE ORGANISMS

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The occurrence of high concentrations of a PCB (Aroclor 1254) in the Pensacola estuary prompted field and laboratory studies by the Gulf Breeze Environmental Research Laboratory (EPA). Monitoring of the estuary indicates the chemical is present in all components—particularly in sediments and fishes. Residues appear to be diminishing in sediments. Toxicity tests show estuarine species sensitive at ppb concentrations in water, with a ciliate protozoan (*Tetrahymena pyriformis* W), shrimps (*Penaeus duorarum*, *P. aztecus*, and *Palaemonetes pugio*), and a fish (*Fundulus similis*), affected at or near 1.0 ppb. Tissue concentrations of Aroclor 1254 similar to those found in natural populations of shrimps from the contaminated estuary were successfully duplicated in laboratory experiments. Shrimps also concentrated the PCB from very low concentrations (0.04 ppb) in the water. Three estuarine species demonstrated pathologic changes at tissue and cellular level after chronic exposure to the chemical. Oysters (*Crassostrea virginica*) developed abnormal infiltration of leukocytes in the connective tissue, spot (*Leiostomus xanthurus*) developed fatty changes in their livers, and shrimp (*Penaeus duorarum*) developed crystalloids in hepatopancreatic nuclei.

Polychlorinated biphenyl (PCB) residues were found in water, sediments and biota of Escambia Bay, Florida, in April 1969 (Duke *et al.* 1970). Subsequently, investigation into the effects of this chemical on estuarine organisms has been a major research goal of the Gulf Breeze Environmental Research Laboratory. In this report, we present data on the chemical in water, sediments and biota of Escambia Bay and adjacent areas, review toxicological data and present some physiological-pathological information. Experimental methods and materials used as well as chemical analyses are given elsewhere (Duke *et al.* 1970, Cooley *et al.* 1972, Hansen *et al.* 1971, Nummo *et al.* 1971a, Lowe *et al.* 1972).

®In this paper, Aroclor and PCB are used interchangeably for Aroclor 1254. Aroclor® is a registered trademark of the Monsanto Company, St. Louis, Mo. Reference to commercial products does not constitute endorsement by the Environmental Protection Agency.

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PCB in Escambia Bay

Unlike other studies in which PCBs have been found in the environment, the chemical in Escambia Bay apparently came from a single point-source and has been identified as Aroclor 1254. Figure 1 is a comparison of chromatograms of (a) an Aroclor 1254 standard, (b) Aroclor 1254 in oysters from a 72-week chronic exposure to the chemical in the laboratory, and (c) PCB isolated from oysters taken from Escambia Bay. PCB concentrations in both oyster samples were similar and both samples and standard were analyzed on the same chromatograph. In laboratory studies with Aroclor 1254, the oysters were continuously exposed to approximately ten ppt for 72 weeks and the similarity of tissue residues to the PCB found in oysters from Escambia Bay indicates that the chemical in the bay has changed little with time.

Monitoring data for the period September 1969 through December 1971 are presented in Table I. Average concentrations in water are given by assuming non-detectable levels as

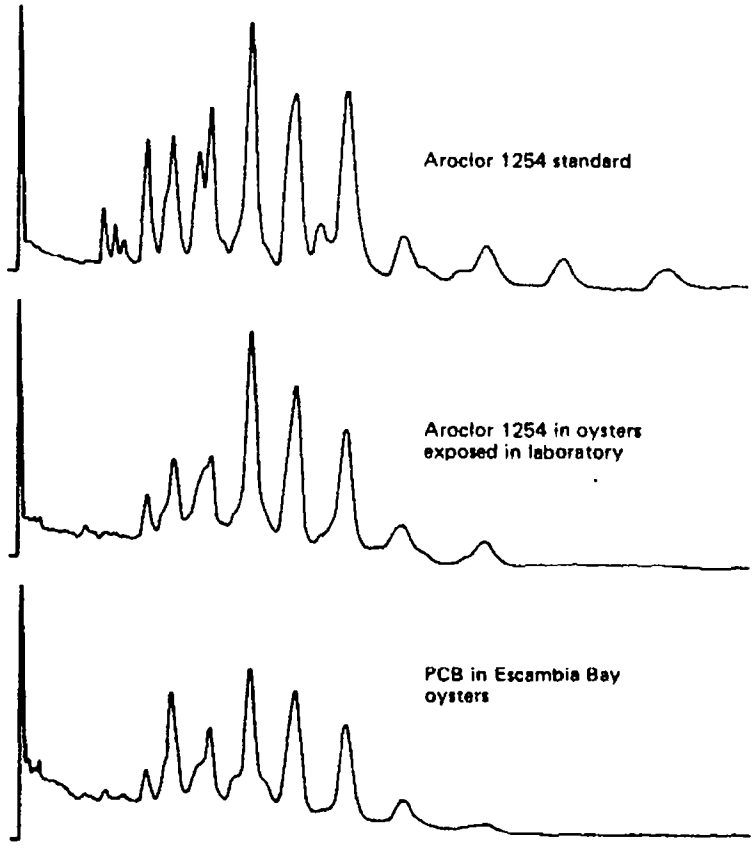


Fig. 1. Chromatograms of an Aroclor 1254 standard, Aroclor 1254 in oysters from a 72-week chronic exposure in the laboratory and PCB in oysters from Escambia Bay.

zero. Concentrations of Aroclor in unfiltered water samples from Escambia River downstream averaged 0.6 ppb. Aroclor was found in 64% of the water samples from the river and in 27% of the water samples from Escambia Bay. The average concentration in sediment samples from the bay was 2.3 ppm. PCB in 101 samples of invertebrates, predominately mollusks and crustaceans, averaged 0.8 ppm. Fishes from the bay had five times as much PCB in their tissues as did invertebrates.

Aroclor in sediment samples from a survey taken in February 1970 were all in the ppm range (Nimmo *et al.* 1971b). The samples were taken from the upper strata (e. g., upper ten in.) by corer or by dredge. In this survey, the maximum residue (61 ppm) observed in the river was found at the outfall from the industry; the maximum in Escambia Bay (30 ppm) was found near the mouth of the river.

The amounts of PCB in sediment samples from several locations within the river and bay have decreased in the ensuing months. The number of samples was inadequate for absolute comparison, but the data indicate a trend. A decrease is especially noticeable in the December 1970 and October 1971 surveys (Fig. 2), in which sediment samples were taken with a corer at three locations in the bay. Generally, residues in the 1971 survey were about one-tenth the 1970 values, except one sample taken below the outfall in the surface stream. PCB in the lower strata (4-12 inches) in the 1971 survey was non-detectable, except at the outfall of the industry. Later, we examined a deeper core–to 24 inches–taken above the trestle but found no residues. Cores taken in the 1971 survey generally indicated less PCB than in 1971.

Whole-body residues of Aroclor 1254 found in shrimps from Escambia Bay and contiguous waters during 1969/1970 are shown in Figure 3. Each datum represents a composite sample of at least five individuals. We show these data to indicate the dispersal of

Table 1. *Concentration of Aroclor® 1254 in water, sediment, and biota, September 1969 through December 1971, in Escambia Bay, Florida*

Samples			Concentration	
Type	Total No.	Positive %	Average (ppm)	Range (ppm
Water	67	64	0.0006	ND- 0.008
Water	37	27	ND	ND- 0.000
Sediment	56	78	2.33	ND-30.
Invertebrates	101	92	0.81	ND- 6.9
Fishes	17	100	3.99	0.29-20.

ND = Non-detectable: Water, < 0.00003 ppm; sediment or biota, < 0.01 ppm.

the chemical in an estuarine environment from an apparent point source by either biological or physical transport. Although the material was originally localized in the sediments of upper Escambia Bay, shrimps captured in lower Pensacola Bay also contained significant amounts of the PCB. In contrast, shrimps from adjacent bays such as Perdido or Choctawhatchee did not have detectable levels of PCBs.

The amounts of Aroclor in biota from the estuary remain relatively high and the latest survey showed amounts generally increasing at higher trophic levels (Fig. 4). In the survey of October 1971, we found no detectable PCB in the sea grasses, *Spartina* sp. and *Zostera marina*. The mollusk, *Neritina reclivata*, contained 0.49 ppm. Of two crustaceans, blue crabs (*Callinectes sapidus*) had the greater residues (6.9 ppm). Among fishes, one might expect sand seatrout (*Cynoscion arenarius*) and Atlantic cutlassfish (*Trichiurus lepturus*) to have the highest residues because these species are predators; instead, the highest residue (10 ppm) was found in silversides (*Menidia beryllina*), a species whose diet consists mainly of plankton.

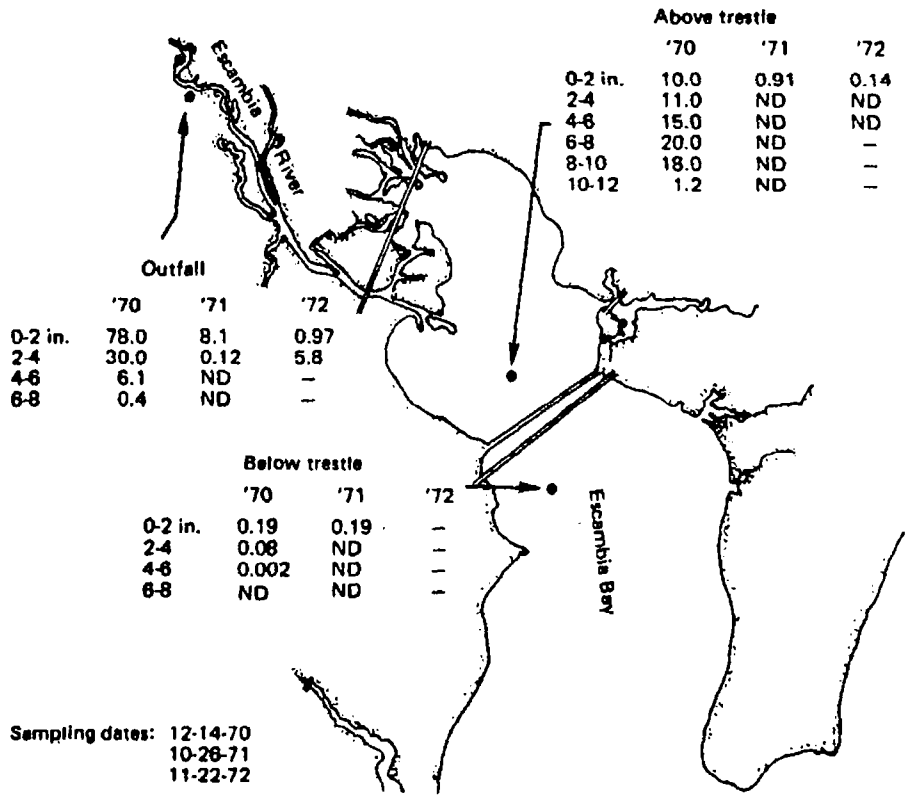


Fig. 2. Comparison of concentrations of Aroclor 1254 in cores taken 10 months apart in upper Escambia Bay and River. Concentrations given in 1971 above and below trestle are averages of two cores each.

In Figure 4, PCB residues are compared in the same species or in species occupying similar trophic levels and captured on the same day in Escambia and East Bays. The collecting site in East Bay is about 35 km from the original source of the chemical. PCB residues in species from Escambia Bay were five to ten times greater than those found in East Bay but the data clearly show that the chemical was found in species captured distant from the original source of the PCB.

Toxicity of Aroclor 1254 to estuarine organisms

Laboratory research on the toxicity of PCB to estuarine organisms began immediately after the chemical was discovered in the Bay. Animals from several trophic levels have been tested.

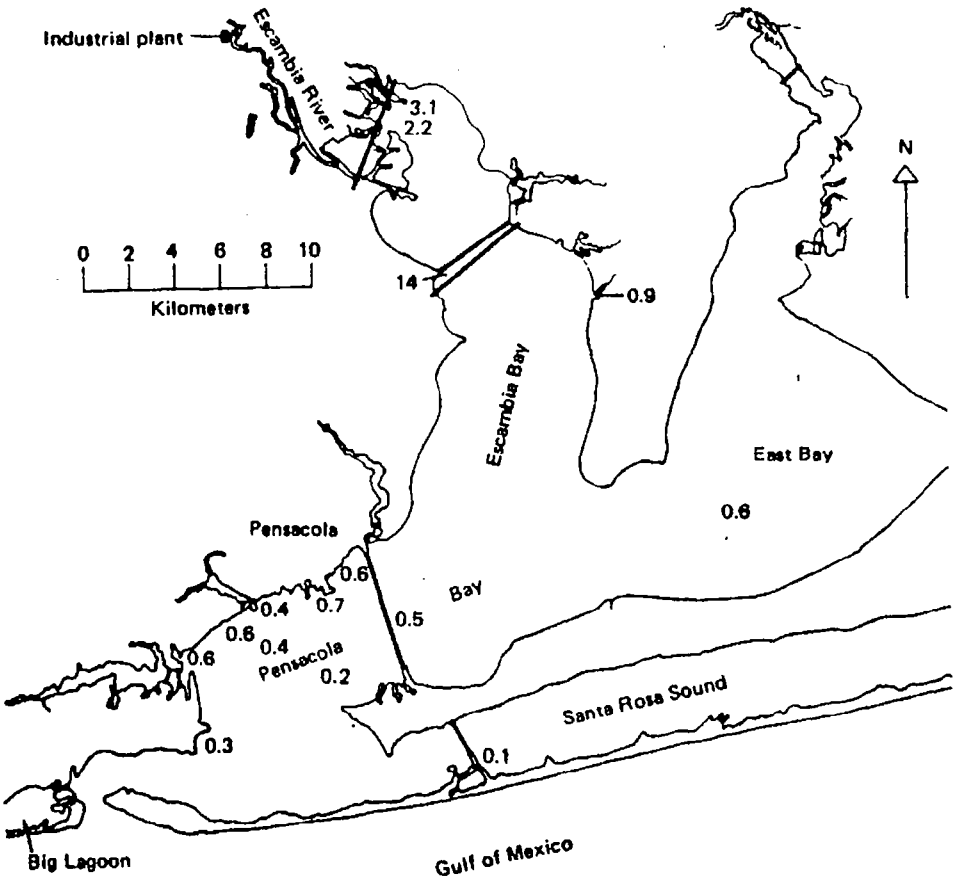
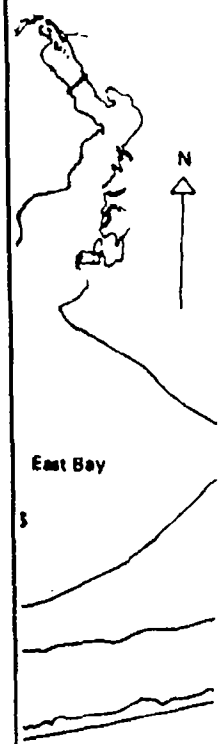


Fig. 3. Residues of Aroclor 1254 in shrimp (whole body) from Escambia Bay and contiguous waters during 1969/1970. Each datum represents a composite sample of at least five individuals.

Population growth in test-tube cultures of the ciliate protozoan, *Tetrahymena pyriformis* W, was reduced significantly by exposure to one ppb of Aroclor 1254 (Table II). Reduction was measured as effect on population growth rate and on population density at 96 hr. Growth rate was estimated as the quantity b of the least squares estimate of the line $y = a + bx$ for the exponetial growth phase of the population growth curve. These ciliates accumulated the PCB from the test media during the exposures. Cells contained a maximum of 60 ppb (dry-weight basis) of PCB when grown for seven days in medium that contained one ppb of Aroclor. Because the ciliates can accumulate PCB from a culture medium, they could be a step in the transport of the chemical into aquatic food webs under natural conditions.

Bioassays were conducted in flowing water to determine the toxicity of Aroclor 1254 to a mollusk, three crustaceans and three fishes (Table III). Growth in oysters (*Crassostrea*



Escambia Bay (ppm)		East Bay	
ND	Spartina	ND	(ppm)
ND	Zostera	ND	
0.49	Olive Nerite	ND	
ND	Rangia	ND	
0.98	Penaeid Shrimp	Trace	
6.90	Blue Crabs	0.46	
3.00	Bay Anchovy	0.68	
3.80	Catfish	0.58	
10.00	Tidewater silversides	0.95	
4.50	Silver perch	0.48	
1.50	Sand seatrout	–	
–	Spotted seatrout	0.12	
1.80	Spot	Trace	
1.60	Atlantic croaker	Trace	
1.30	Hogchoker	ND	
2.90	Atlantic cutlassfish	0.72	

Fig. 4. Comparison of concentrations of Aroclor 1254 found in species collected in Escambia (left side) and East Bays (right side): seagrasses, *Spartina* sp. and *Zostera marina*; Rangia clams, *Rangia cuneata*; olive nerite, *Neritina reclivata*; brown and white shrimp, *Penaeus aztecus* and *P. setiferus*; blue crabs. *Callinectes spidus*; bay anchovy, *Anchoa mitchilli*; sea catfish and gafftopsail catfish, *Arius felis* and *Bagre marinus*; tidewater silversides, *Menidia beryllina*; silver perch *Bairdiella chrysura*; sand seatrout, *Cynoscion arenarius*; spotted seatrout, *Cynoscion nebulosus*; spot, *Leiostomus xanthurus*; Atlantic croaker, *Micropogon undulatus*; hogchoker, *Trinectes maculatus*; and Atlantic cutlassfish, *Trichiurus lepturus*.

virginica), exposed to five ppb for 24 weeks was significantly reduced, but growth in oysters exposed to one ppb for 30 weeks was not. Earlier work showed that hydrocarbon compounds inhibited shell deposition significantly at concentrations of 0.1 to 0.5 ppm in short-term tests (Butler 1966). In tests lasting about two weeks, various shrimps

Table II. *Effect of Aroclor 1254 on population growth of* Tetrahymena pyriformis *W* [a]

Toxicant (μg/liter)	Mean growth rate[b] *b*	Difference (%)	Mean population density[b] (absorbance)	Difference (%)
0	0.0212		1.044	
0.1	0.0203	− 4	0.984	− 6
1.0	0.0195	− 8[c]	0.936	− 10[d]
10.0	0.0199	− 6[c]	0.954	− 9[d]

[a] After Cooley *et al.* (1972).
[b] Means of 6 replicate experiments.
[c] $F(3, 15) = 6.00$ ($P < 0.01$).
[d] $F(3, 15) = 23.001$ ($P < 0.005$).

Table III. *Chronic toxicity of Aroclor® 1254 to estuarine animals in flowing water, 1 to 30 weeks* [a]

Test animals	Concentration, ppb (μg/l)	
	Range	Minimum affecting
Pink shrimp	0.6- 19.0	0.9
Longnose killifish	1.0-100.0	1.0
Grass shrimp	0.2- 12.5	1.3
Brown shrimp	0.1- 1.4	1.4
Pinfish	5.0	5.0
Spot	1.0- 5.0	5.0
Eastern oyster	1.0- 5.0	5.0

[a] Controls did not exceed 25% mortality. Toxicity in oysters was measured by reduced shell growth.

(*Penaeus duorarum*, *Penaeus aztecus*, and *Palaemonetes pugio*) were killed by exposure to 0.9, 1.4, and 4.0 ppb, respectively. In tests lasting two weeks or longer this chemical was lethal to longnose killifish (*Fundulus similis*) at 1.0 ppb, and pinfish (*Lagodon rhomboides*) and spot (*Leiostomus xanthurus*) at 5.0 ppb. Test animals exposed for over one week accumulated the PCB from the water; concentrations ranged from about 10^4 in crustaceans and fishes to 10^5 in oysters over water concentrations.

Acute toxicity tests did not show the true sensitivity of marine species to this compound. In comparison to short-term tests lasting 48 hr, Aroclor in chronic bioassays lasting one week or more proved to be 100 times more toxic.

Mortalities were "delayed" as they usually did not begin until after one week of exposure and continued to occur after the animals were removed from the toxicant. This delayed mortality was similar to that observed with the insecticide mirex (Lowe *et al.* 1971). Gross signs of poisoning varied with species. Fishes typically developed hemorrhagic lesions on the body, ragged fins, and stopped feeding. Shrimps became lethargic and also stopped feeding, thereby mimicking the effects of low concentrations of DDT [1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane] (Nimmo and Blackman 1972). Shrimps appear to be most susceptible to the chemical during molting, as previously noted by Duke *et al.* (1970) and Wildish (1970).

Accumulation of Aroclor 1254 by shrimp

The pathway by which organisms obtain toxicants or the actual effects observed in the laboratory under controlled conditions may or may not approximate those obtained under field conditions. The pathway appears to be an open question in the field of aquatic toxicology and the need for such research was stated by Sodergren *et al.* (1972) after a study of the accumulation of DDT and PCB by a crustacean.

If we could produce tissue distributions of PCB in shrimp in the laboratory similar to those found in the field, some insight might be gained into its mode of entry and concentrations in food or water in nature. We began a study by determining PCB residues of shrimp from Escambia and Pensacola Bays (Fig. 5). We administered PCB at three concentrations (0.2, 0.68 and 43 ppm) in food in aquaria with flowing PCB-free seawater (Fig. 6). PCB was added at 3.0 ppb to seawater filtered through gravel and charcoal and 3.0 ppb was added to unfiltered seawater (Fig. 7). PCB was added to unfiltered seawater at 3.5 and 0.2 ppb (Fig. 8). Analytical methods for PCB were those of Nimmo *et al.* (1971a). The results of the field surveys and laboratory studies are expressed as the "relative concentration" (Fig. 5, 6, 7, 8):

$$\text{Relative concentration} = \frac{\text{ppm in a single tissue or organ}}{\text{ppm in all tissues or organs}} \times 100.$$

The proportion of Aroclor found in tissues of shrimp exposed in the laboratory to 0.2 ppb in water was nearest to that found in feral shrimp captured in the bays (Fig. 8) and concentrations were within the ranges found in shrimp from the field. Thus, we believe

that shrimp from the laboratory exposures or feral shrimp from the bays probably obtained most of the chemical from water. Shrimp could have absorbed PCB from the water column near the sediment-water interface or directly from interstitial water of the substrate. However, this suggestion does not exclude the possibility that shrimp obtained some of the chemical from food, as was the case observed in Fig. 6.

We believe the rate of Aroclor loss from shrimp was low and insufficient to affect the localization of Aroclor in the tissues of shrimp because in earlier experiments, we found that in the hepatopancreas only half of the chemical was lost in 17 days and in the remaining tissues, Aroclor content remained constant for five weeks (Nimmo *et al.* 1971a).

The shrimp used in the laboratory exposures and those captured in the bays are all benthic species—either feeding on sediments or burrowing directly in them. It seems reasonable to suppose that adsorption of PCB directly through the gills from contaminated sediments would be greater because the animals are continually moving PCB-contaminated water through them. The results of the laboratory studies lead us to believe that concentrations of PCB available to shrimp in Escambia and Pensacola Bays were low (e.g., <1.0 ppb in water; <1.0 ppm in food).

To determine if there was a concentration below which shrimp could not accumulate the chemical, we tested several hundred grass shrimp (*Palaemonetes pugio*) at 0.04, 0.09

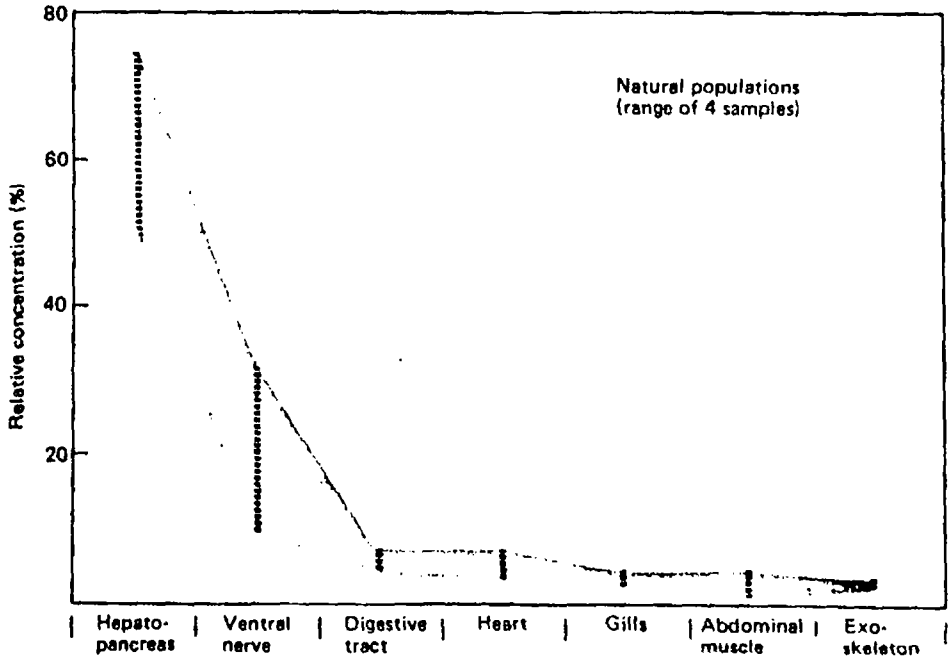


Fig. 5. Distribution of Aroclor 1254 in tissues of shrimp expressed as relative concentration (%). The grey area represents the range of concentrations in four composite samples of shrimp from different locations in the Pensacola estuary on different dates.

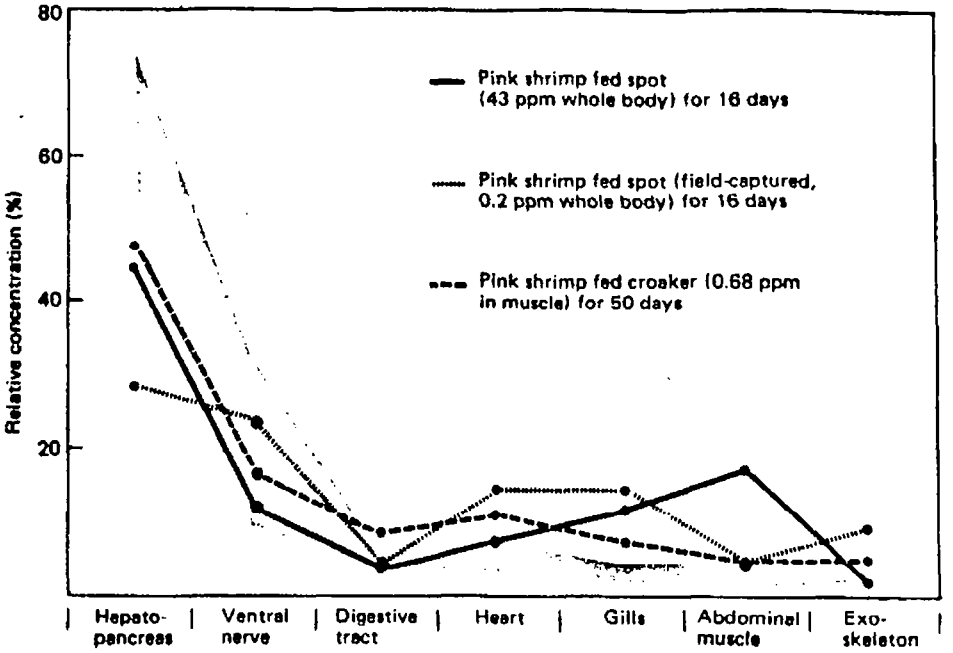


Fig. 6. Distribution of Aroclor 1254 in tissues of shrimp fed Aroclor 1254-contaminated diets. The grey area is as in Fig. 5.

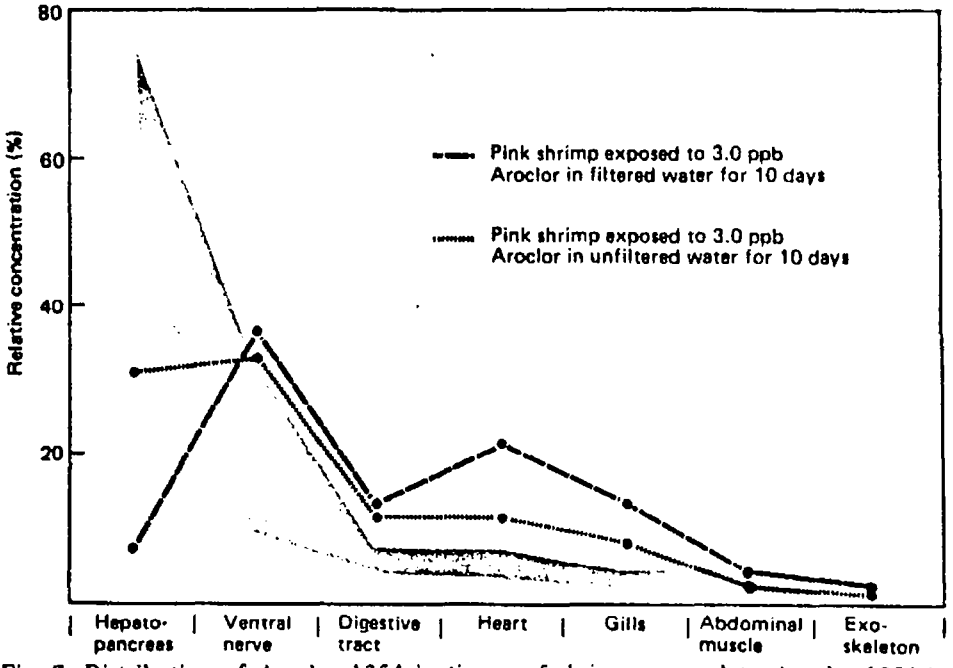


Fig. 7. Distribution of Aroclor 1254 in tissues of shrimp exposed to Aroclor 1254 in filtered and unfiltered seawater. The grey area is as in Fig. 5.

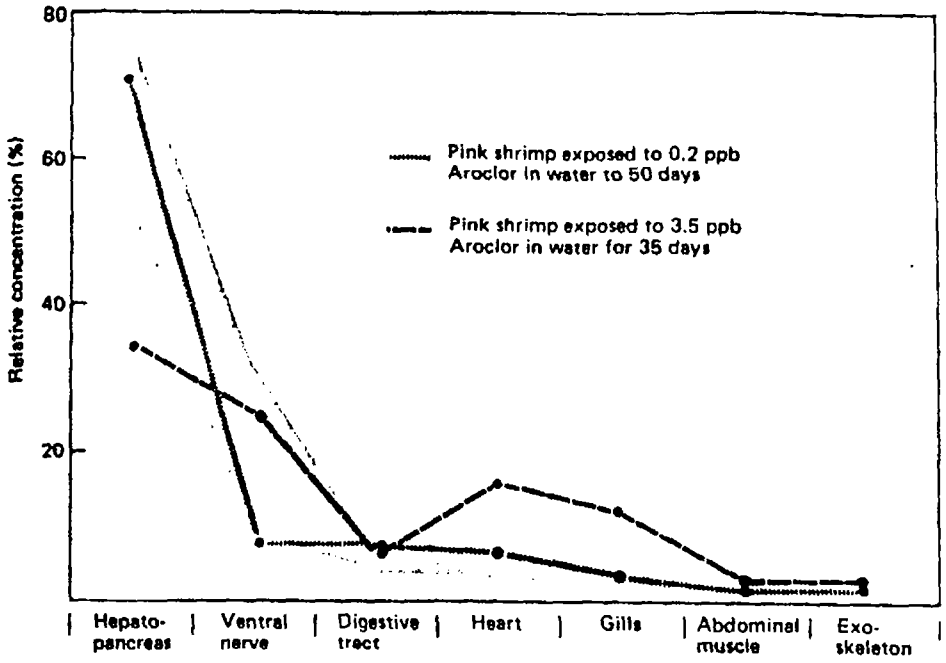


Fig. 8. Distribution of Aroclor 1254 in tissues of shrimp exposed to the chemical in flowing-water aquaria. The grey area is as in Fig. 5.

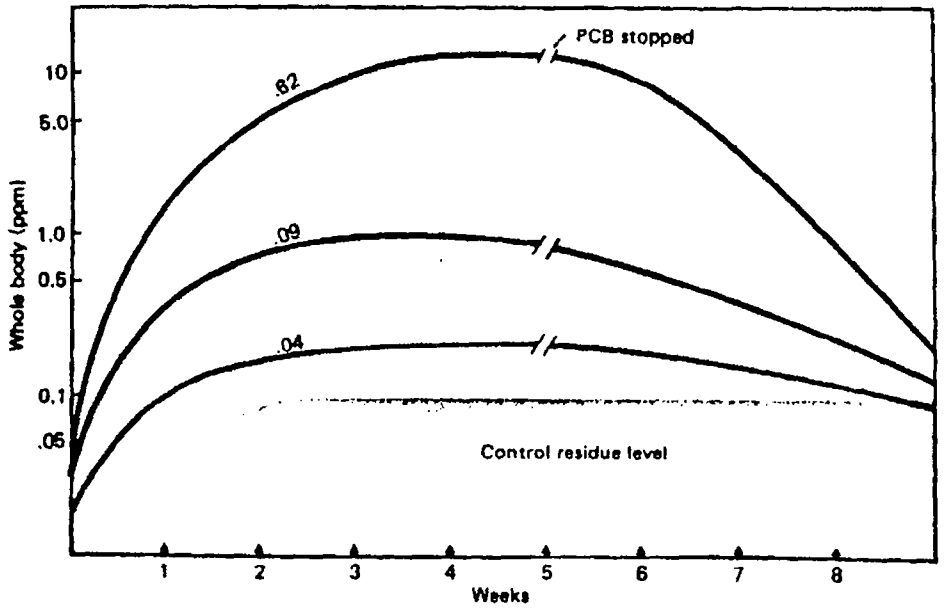
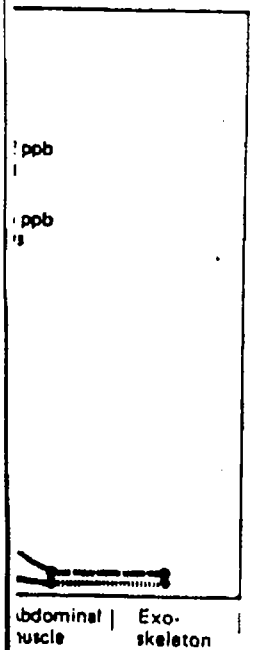


Fig. 9. Aroclor 1254: Uptake and depuration in grass shrimp exposed to 0.04, 0.09 and 0.62 ppb in water.



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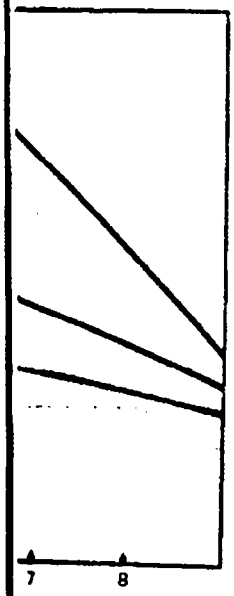

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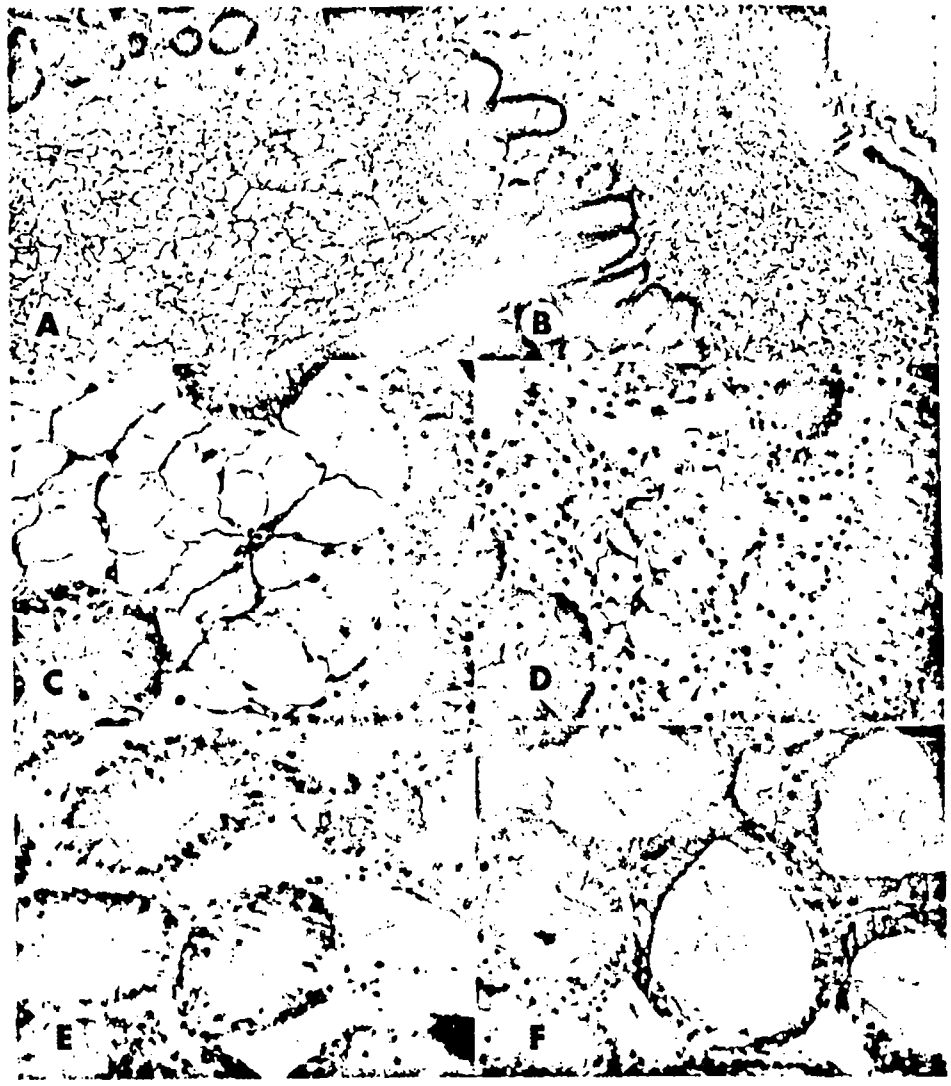

Fig. 10. (A) Normal vesicular connective tissue (parenchyma) from control oyster. Note uniform cell patterns and distribution of leukocytes. X100. (B) Vesicular connective tissue from oyster exposed to PCB for six months. Note loss of uniform cell distribution and infiltration by many leukocytes. X100. (C) Normal vesicular connective tissue from control oyster. X450. (D) Tissue from exposed oyster. Note many leukocytes and degeneration of vesicular connective tissue adjacent to gut epithelium. X450. (E) Normal digestive gland tubules from control oyster. Note the thick epithelia which form normal triradiate lumina. X450. (F) Digestive gland tubules of oyster exposed to PCB. Note atrophy (thinning) of tubule epithelium and enlarged, abnormal lumen of tubule X450.

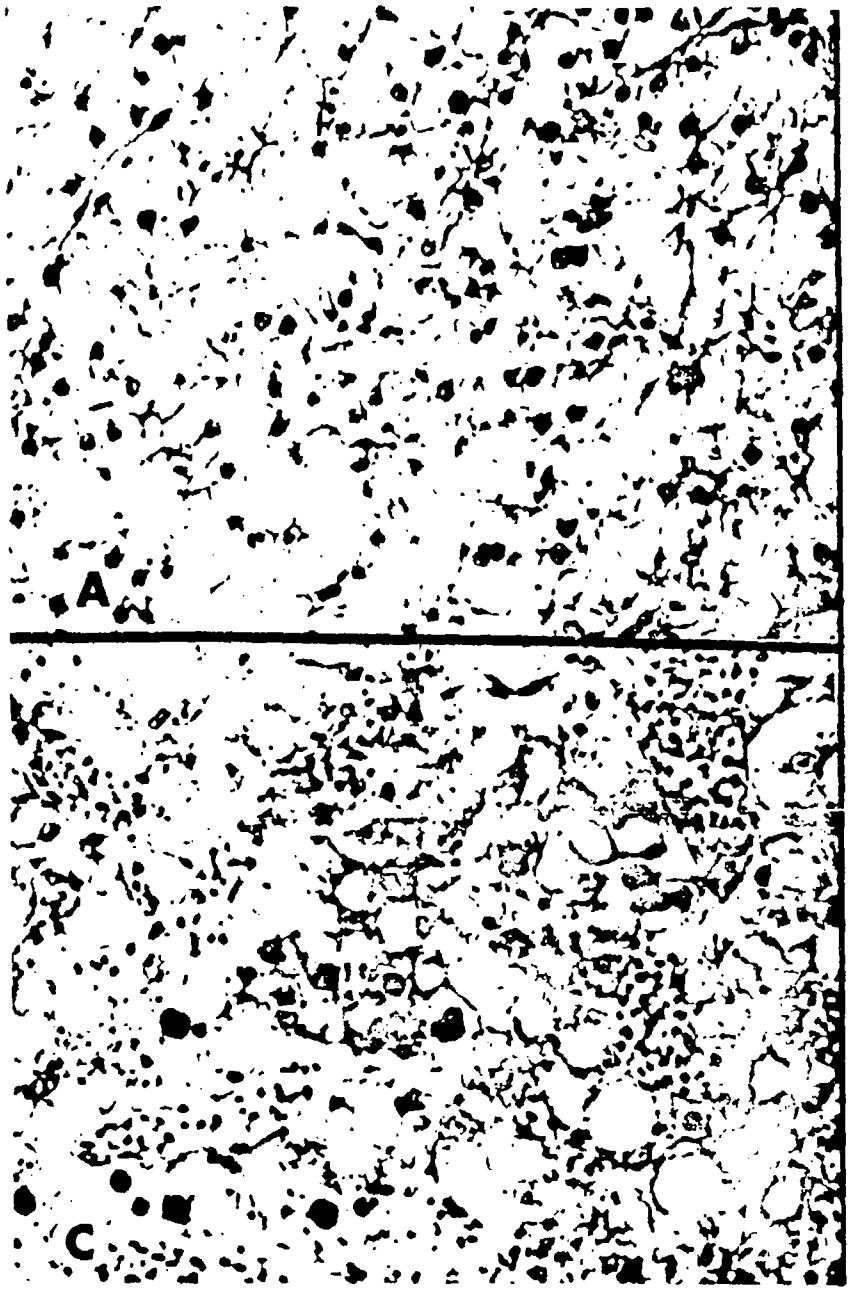

Fig. 11. (A) Liver parenchyma of normal spot (*Lagodon rhomboides*) from control tank. Note uniform orientation of hepatocytes. X1000. (B) Liver parenchyma of spot exposed to PCB for several weeks, intermediate pathogenesis. Note large, smooth-edged vacuoles indicative of abnormal fatty-change in hepatocytes. X1000. (C) Liver parenchyma of spot exposed to PCB until moribund, advanced pathogenesis. Note large vacuoles, amor-

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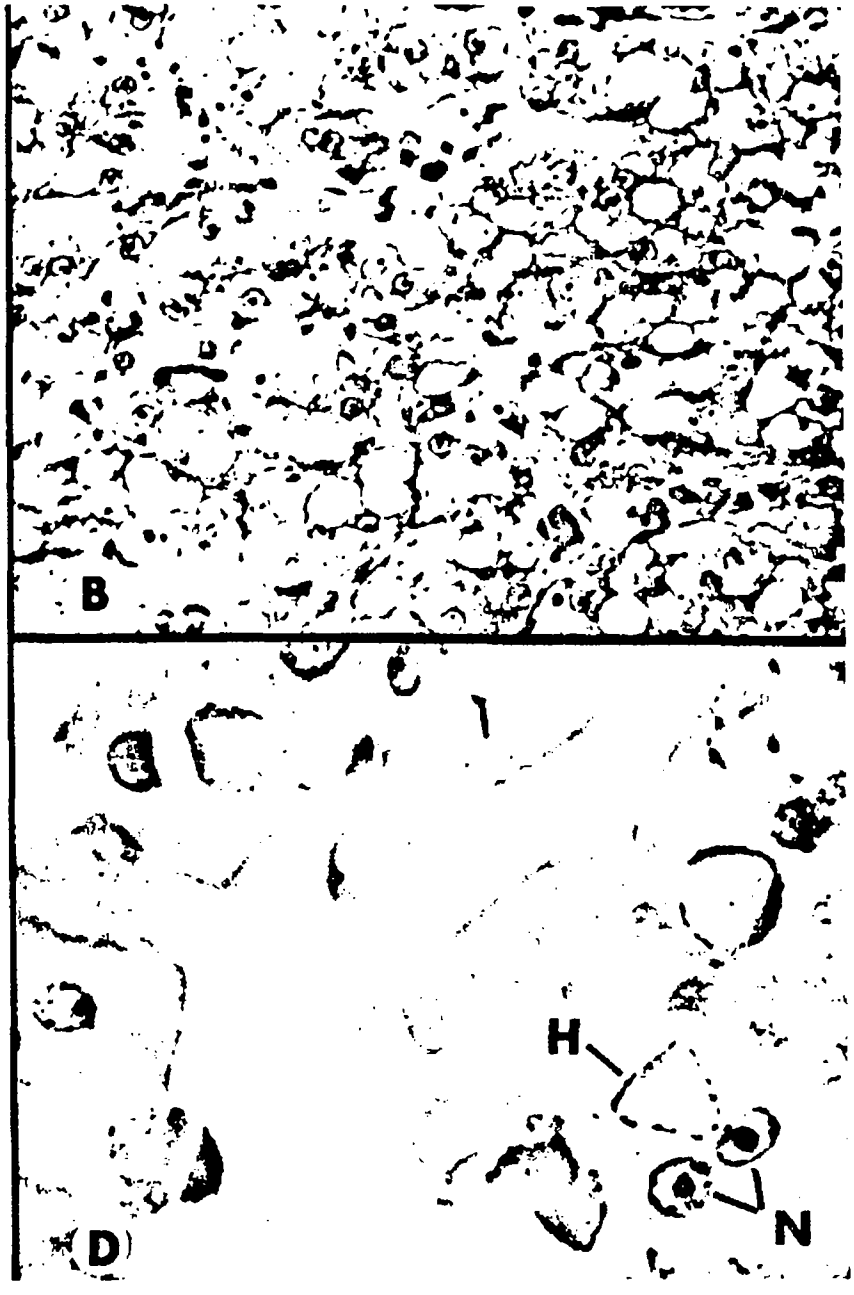

phous inclusions and sinusoidal congestion. X1000. (D) Hepatopancreas (digestive gland) tubule from pink shrimp exposed to PCB. Note triangular crystalloids in some hypertrophied nuclei (H); also, normal nuclei (N) with large, prominent endosomes. (Feulgen reaction; DNA appears black in photomicrograph). X1000.

Fig. 12. (A) Fresh squash of hepatopancreas from exposed shrimp. Note two crystalloids in center. X1000. (B) Single, large crystalloid from fresh squash of hepatopancreas of exposed shrimp. X1000. (C) Longitudinal section of hepatopancreatic duct with branching tubules. In exposed shrimp, the crystalloids appear in the tubule epithelia nearer the main hepatopancreatic ducts. X1000. (D) Cross-section of hepatopancreas tubule from exposed shrimp. Note crystalloids in several epithelial nuclei; also, normal nuclei with conspicuous endosomes. X450. (E) Intermediate size crystalloid within hypertrophied epithelial nucleus. X1000. (F) Pathologic effect of crystalloid in PCB-exposed shrimp. Note rupture of cells and nuclei releasing the crystalloids. X1000.

and 0.62 ppb of Aroclor 1254. The tests were conducted in flowing-water aquaria as before, with one modification. Each tank had a false floor of nylon screen to hold the animals above the detritus. We believe the shrimp obtained more chemical through absorption from the water than in previous experiments.

Within the test concentrations, no threshold level existed below which shrimp did not accumulate the chemical (Fig. 9). Concentrations produced in the shrimp (whole-body) were about 0.2, 1.0 and 10 ppm, respectively, and these were reached between the third and fifth weeks of exposure. Concentrations in the shrimp did not reach equilibrium during the five-week exposure but the rate of accumulation decreased with time. When transferred to PCB-free water, the shrimp lost most of the chemical in four weeks.

Pathology in estuarine organisms

To date, toxicology of PCBs with respect to histopathological effects in estuarine organisms has been little studied. Published sources of information are from studies on mammals and birds (Dahlgren *et al.* 1972, Vos 1972, Norback and Allen 1972) and a single study on fishes (Couch 1972). Structural changes found in tissues of oysters, fish, and shrimp exposed to Aroclor 1254 are characterized below.

Oysters exposed to 5.0 ppb of Aroclor 1254 for up to six months showed several major tissue changes. Normal structural pattern of oyster vesicular connective tissue (parenchyma) is seen in Figure 10A, and the irregular and broken pattern representative of altered tissue from exposed animals is seen in Figure 10B. In exposed oysters, an abnormal infiltration of leukocytes was found in the vesicular connective tissue (Fig. 10C and 10D). Sections of digestive glands of normal control oysters (Fig. 10E) can be compared to those in exposed oysters (Fig. 10F). The epithelia of distal digestive tubules of exposed oysters have undergone atrophy and surround enlarged lumina. Oysters that were removed from Aroclor-contaminated water and allowed to live in natural water for several weeks demonstrated partial or complete tissue recovery.

Spot, an estuarine fish, exposed for two weeks or longer to 5.0 ppb of Aroclor, showed fatty changes in their livers. Normal liver tissue has regular distribution of hepatic cells and typical nuclei (Fig. 11A). Note the regularity in the orientation of liver cords, cells and uniform scattering of nuclei. In intermediate stages of liver pathogenesis in experimental fish, there are extreme fatty changes characterized by the presence of large vacuoles within hepatocytes and disorientation of liver cord distribution (Fig. 11B). Figure 11C shows an advanced stage of pathogenesis in a moribund fish. Note the presence of intracellular PAS-positive bodies (ceroid) and congestion of blood sinuses, and severe vacuolation.

Probably the most dramatic tissue change associated with chronic PCB exposure was observed in shrimp. The normal hepatopancreas, or digestive gland, of shrimp is a tightly packed organ of small elongated tubules (Fig. 12C). Figure 12D shows a cross section through one of these tubules. In the hepatopancreas of exposed shrimp, pyramidal

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crystalloids of various sizes were found as inclusion bodies in the nuclei of epithelial cells (Fig. 12D and 12E). Free crystalloids are shown in Figure 12F. We know of no other report of the occurrence of precisely shaped crystalloids in hepatopancreatic tissue of crustaceans. We have routinely studied unfixed, fresh exposed shrimp and have found crystalloids in squashes of the tissue (See Fig. 12A and 12B).

Epithelial cells of the hepatopancreas from shrimp which were exposed to three ppb Aroclor for at least 30 days are shown in Figure 11D. Exposed shrimp that do not have crystalloids have no conspicuous pathologic tissue signs. In those that have the crystalloids, hypertrophy of the affected nucleus results. Eventually, the growth of the crystalloid inclusion distorts and ruptures the nuclear membrane. Several nuclear membranes and inclosed crystalloids are indicated in Figure 11D. The crystalloids are histochemically positive for protein. They occur in widely separated nuclei but may also appear in clusters of adjacent nuclei and are most abundant in epithelial cells of tubules proximal to the main hepatopancreatic ducts.

These crystalloids were found in individual shrimp before moribundity or death. In certain exposures, crystalloids have been found in up to 80% of the survivors but the incidence is very low over time until about 75% of the test animals have died. Crystalloids were found more often in larger shrimp than in juveniles. At present, we are attempting to establish whether crystalloids occur in individuals from other localities when exposed to PCB, in other species of shrimp, or in shrimp from contaminated areas in nature.

Several possibilities exist as to the origin of the crystalloid inclusions. One suggestion was that they may be the result of sequestering of some normal or abnormal metabolite. Another possibility is that they represent a material produced by a virus[2] and were produced under PCB stress. In reference to this suggestion, Friend and Trainer (1970) showed that PCB enhanced the pathogenic effects of hepatitis virus in ducks.

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[2]One of us (J. C.) as a result of electron microscope studies has recently found rod-shaped virus-like particles occluded within the crystalloid inclusion bodies. This matter is currently under study.

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