DDT and PCB in Marine Animals from Swedish Waters

by

S. JENSEN

Institute of Analytical Chemistry, Stockholm

A. G. JOHNELS
M. OLSSON

Swedish Museum of Natural History, Stockholm

G. OTTERLIND

Institute of Marine Research, Lysekil

Analyses of pesticide residues in a wide range of marine organisms from the coastal waters of Sweden show that there is a marked contamination in the Baltic. There are signs of an increase in polychlorinated biphenyls (PCB) from north to south in this area. Exceptionally large amounts of residues were found in white tailed eagles from the archipelago of Stockholm.

CHLORINATED pesticides can spread in living matter, and they are now present all over the world. In 1966 (ref. 1), it was discovered that some previously unknown substances isolated in analyses of pesticide residues were polychlorinated biphenyls (PCB). In Sweden, they occur in the natural environment in the same amounts as the chlorinated pesticides[1,2]. Recently, PCB has been found in organisms in the Netherlands, Great Britain[3-5] and the United States[6]. PCB is used almost exclusively in industry, and it seems to be more persistent than DDT. We have assessed PCB and DDT (including metabolites) contamination in Swedish marine ecosystems by analysing 176 samples. The hydrographical conditions along the Swedish coasts are not uniform so the residues are classified according to the four regional subdivisions shown in Fig. 1. Few samples were taken from the Sound and so this area has been included in the Baltic proper.

The species studied were: (1) mussel (*Mytilus edulis*); (2) herring (*Clupea harengus*); (3) plaice (*Pleuronectes platessa*); (4) picked dogfish (*Squalus acanthias*); (5) cod (*Gadus morhua*); (6) salmon (*Salmo salar*); (7) grey seal (*Halichoerus grypus*); common seal (*Phoca vitulina*) and ringed seal (*Pusa hispida*); (8) guillemot (*Uria aalge*); (9) white tailed eagle (*Haliaeetus albicilla*); (10) eggs of white tailed eagle; (11) heron (*Ardea cinerea*). Three samples of separated fish oil were also analysed. Two were taken from the Norway pout (*Boreogadus esmarki*) and one from herrings from the northern part of the west coast. One sample (largely herring oil) was also extracted from fish from the southern Baltic proper. This was unseparated.

Homogenates of one or more mussels were analysed. In the fish, axial muscle tissue extracted from the dorsal side above the lateral line, approximately a third of the length from the tail was analysed. In nine out of eleven salmon, the samples had to be taken immediately behind the head (only heads were available). Most of the samples from the seals were taken from the blubber in the tail. In two seal pups from the Gulf of Finland, the samples were from the fore leg, and in one grey seal from the Baltic proper, the sample was liver. It is reported[3], however, that in seals the chlorinated hydrocarbon content in the fat is approximately constant throughout the different parts of the body. As a rule, the specimens, or parts of the specimens, were sent to the Swedish Museum of Natural History immediately after capture and were stored at $-20°$ C. Three seal tails from the archipelago of Stockholm and one from the Gulf of Bothnia were, however, kept by the hunter for six months in a non-heated room. In the homogenized eggs of the guillemot the fat varied from 3·6 to 11 per cent probably because homogenization before aliquotation was not quite perfect. Samples were taken from both the pectoral muscle and brain of the white tailed eagle. All the eagles were found dead and in some cases in a state of decay, and all the eggs were either addled, rotten or dry. In the heron, pectoral muscle only was analysed.

DDE was the principal metabolite of DDT found.

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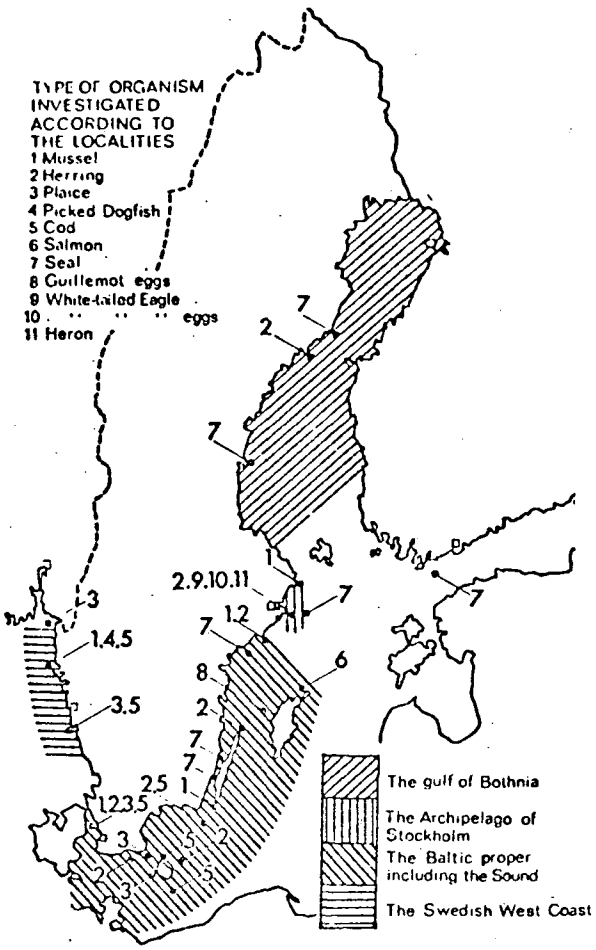

Fig. 1.

DDD was found in small concentrations, not usually more than 10 per cent of the ε DDT, in most samples. (ε DDT stands for the sum of DDT and DDE—DDE was multiplied by 1·11 to correspond to the original DDT.) For seals from the archipelago of Stockholm and for salmon from the Baltic proper which had relatively large amounts of DDD, ε DDT stands for the sum of DDT, DDE and DDD.

We analysed all the samples using gas chromatography and electron capture detection. Confirmations were carried out with double column systems combined with treatments with sulphuric acid and potassium hydroxide[7].

We found that the principal PCB components were PCB numbers 7–14 (low number is equal to a low chlorination degree), but the mussels also contained the lower numbers (the PCB composition being similar to clophen A 50). The PCB amounts given in Table 1 are, nevertheless, the sum of all PCB components found in the samples although the relative amounts of the different numbers vary. The results suggest that the low PCB numbers are metabolized or excreted faster than the higher numbers, so that there is an increase of the latter as it passes through a food chain.

An attempt to quantitate PCB has been made in Sweden[7], but because the method is still rather rough the values may be correct only within a factor of 2. The method is indirect because all the PCB components have not been isolated yet, and it is based on a combination of mass spectrometry, micro-colorimetric and electron capture detection. For each twenty samples a blank on the solvent and a residue free plaice sample with added pesticides (lindane, aldrin, *pp'*DDE, dieldrin, *pp'*-DDD and *pp'*-DDT) were also analysed. Recovery was above 80 per cent, but as normal in residue analysis[8] we made no correction.

The mean figures in Table 1 are calculated from the average value of each locality within the four regions. The residue values are based both on fresh tissue and on hexane extractable fat. The figures for fresh tissue are comparable with reported values from other countries. We believe, however, that the fat values are of particular interest from an ecological point of view. Fat is important in the transport of energy and as a carrier of the residues between the species in the food chain. Furthermore, there is a great seasonal variation in fat content within the populations. The difference between the greatest and the smallest residue level in the extractable fat of individuals of one species at a given time, in a given place, is less than in fresh tissue. The fat content of herring muscle, for example, varied from about 1 per cent in the spring to about 10 per cent in the autumn. Our discussion will therefore be based largely on residue levels in extractable fat.

Mussels from the west coast of Sweden have lower average values of ε DDT and PCB than from the Baltic proper and the archipelago of Stockholm. The level of ε DDT in mussels from the Dutch, West German and British coasts[4,9] has an upper limit similar to that in mussels from the Baltic proper and the archipelago of Stockholm.

DDT contamination in individual plaice, cod and in fish oil is greater in the Baltic proper than off the west coast of Sweden, and there is little or no overlapping of the residue values. In the case of PCB, a plaice from the very polluted Idefjord in the west coast increases the average value for the west coast samples from 0·69 to 5 p.p.m. Other individual samples of fish from the west coast contain PCB residues of the same magnitude as residues in specimens from the Baltic. This may be the result of coastal contamination. The picked dogfish, which lives more in the open sea, seems to have the same amount of ε DDT, but a smaller amount of PCB than other fish from the west coast of Sweden.

The samples of herring from the Baltic proper, the archipelago of Stockholm and the Gulf of Bothnia had large residues of ε DDT. No herring from the west coast of Sweden has been studied. Recently we analysed eight herrings from the west coast. On fat basis the mean values were: ε DDT, 1·9; DDT, 0·86; and PCB, 0·75, in good agreement with the fish oil values (see Table 1). Juvenile herring from the Dutch Wadden Sea (coastal waters)[4,6] contained DDE residues in fresh tissue with an upper limit of the same magnitude as in herring from the Baltic area.

Residues of PCB and ε DDT in one sample of fish oil from the southern Baltic proper were about five to ten times greater than the three samples from the west coast of Sweden. The figures for the latter are similar. This is in accord with residues in fat in fish from the respective areas.

One herring from the Sound had a much larger content of PCB (23 p.p.m.) than the herring from the rest of the Baltic area. This increased the average value in this area from 4·6 to 6·8 p.p.m. Cod and plaice from the Sound also had a larger PCB content than specimens from the Baltic proper and off the west coast. This suggests that there is local contamination of PCB in the Sound. Furthermore, the mean levels of PCB noted in herring were smaller in the Gulf of Bothnia than in the rest of the Baltic.

The vagrant Baltic salmon had about ten times more chlorinated hydrocarbons residue than a sample reported from Great Britain[3].

The seals from the Baltic proper, the archipelago of Stockholm and the Gulf of Bothnia contained large amounts of residues of both ε DDT and PCB, about ten times greater than amounts found in Great Britain[3], Canada[3] and the Netherlands[10]. One specimen from

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the archipelago of Stockholm had 310 p.p.m. in fat, increasing the mean value for this part of the coast from 117 p.p.m. to 170 p.p.m. The levels of PCB decrease from south to north. This fact, together with lower average values of PCB in herring from the Gulf of Bothnia, indicates decreasing PCB contamination from south to north in the Baltic. Seal pups, one week old, also contained large amounts of ε DDT and PCB residues, and seal milk from the stomach of one of these pups had the same level.

Guillemot eggs from the Baltic proper provided additional information on the contamination of the Baltic. The means of ε DDT and PCB in eggs (wet weight) were 40 p.p.m. and 16 p.p.m. respectively, which for ε DDT is about ten times the levels reported from Great Britain[9,11-13]. The Baltic levels, both for ε DDT and PCB, are about four to five times greater than levels reported from the west coast of the United States[4], if the figures for residues in fat are compared. In guillemot eggs from the Baltic, as much as 87 per cent of ε DDT was DDE.

The population of white tailed eagles in Sweden seems to be on the decline. Only about two of ten pairs nesting in the archipelago of Stockholm succeeded in hatching during the past few years (E. Larsson, personal communication). All specimens of white tailed eagle studied up to the present contained at least three accumulating substances known to be toxic. These are DDT metabolites, PCB and mercury compounds[14]. Figures for four birds are reported in Table 1. A fifth specimen is not included because the fat content was not determined, but the pectoral muscle and brain tissue of this bird (wet weight) contained 190 and 39 p.p.m. respectively of DDT (as DDE), and 230 and 24 p.p.m. of PCB. Two specimens from northern Sweden, one of which died by accident, contained 2·6 p.p.m. (1·8–3·4) of DDT (as DDE) and 2·9 p.p.m. (1·8–3·9) of PCB in muscle tissue, the second having 1·8 p.p.m. DDT (as DDE) and 1·2 p.p.m. PCB in brain tissue. The figures from the archipelago of Stockholm are thus a hundred times larger, suggesting that there is a strong contamination in this area.

It is well known that residues of chlorinated hydrocarbons tend to be greater in the higher trophic levels than in the lower. Some simplified food chain relations can be deduced from the figures in Table 1. These are fish to seal, fish to guillemot, fish to heron and fish to white tailed eagle. In all cases, the increase in residues from prey to predator is at least ten-fold in both whole tissue and fat. In the eagle and heron, the increase is up to 100 times; but for both of these species some important food organisms are missing in this investigation. There are indications (Table 1) that the percentage of DDE and of ε DDT increases as it progresses from lower to higher trophic levels. This may be the result of decomposition of DDT within the organisms. In all the samples from birds, the residues are almost exclusively DDE, while in seals and fishes there is up to 50 per cent of DDT. This difference may be the result of differences in metabolism[15].

As previously mentioned, the seals from the archipelago

Table 1. CONCENTRATION OF ORGANOCHLORINE COMPOUNDS IN SWEDISH MARINE ORGANISMS 1965–68

	No. in sample	P.p.m. in fat			P.p.m. in fresh tissue			Per cent fat
		εDDT	DDT	PCB	εDDT	DDT	PCB	
Swedish West Coast								
Mussel	17	1	0·6	2	0·02	0·007	0·084	1·3
Oct. 1966 Dec. 1967		(0·4–5)	(0·3–1·3)	(0·5–7·0)	(0·005–0·04)	(0·002–0·03)	(0·011–0·33)	(0·66–2·6)
Plaice	3	1		5	0·006	0·004	0·021	0·5
Sept. 1966		(0·9–2)	n.e.	(0·4–14)	(0·003–0·009)	(trace–0·006)	(0·002–0·056)	(0·4–0·5)
Cod	4	1		7·3	0·005	0·003	0·019	0·30
Sept. 1967		(0·6–2)	n.e.	(1·8–16)	(0·001–0·006)	(n.d.–0·006)	(0·006–0·030)	(0·19–0·34)
Picked dogfish	7	1·5	0·91	1·5	0·15	0·091	0·15	9·6
Aug. 1968		(0·29–3·9)	(0·15–2·3)	(0·81–2·4)	(0·028–0·33)	(0·015–0·21)	(0·054–0·30)	(6·7–14)
Fish oil	3	2·1	1·2	0·74				100
Oct. 1968		(1·5–2·6)	(0·83–1·4)	(0·54–1·0)				
Baltic Sea proper incl. the Sound								
Mussel	40	6	1·8	4·3	0·03	0·02	0·03	0·92
Oct. 1966, Dec. 1966 Dec. 1967, Jan. 1968		(0·9–10)	(0·5–2·9)	(1·9–8·6)	(0·009–0·07)	(0·003–0·023)	(0·008–0·057)	(0·46–1·6)
Herring	18	17	0·7	6·8	0·68	0·40	0·27	4·4
April, Sept. 1966–68		(4·1–37)	(1·5–21)	(0·5–23)	(0·093–2·3)	(0·012–1·3)	(0·000–1·0)	(0·7–12)
Plaice	6	2·7	2·1	2·7	0·018	0·013	0·017	0·65
Sept. 1967		(1·4–7·8)	(0·6–7·2)	(1·7–4·8)	(0·006–0·036)	(0·003–0·029)	(0·010–0·032)	(0·58–0·71)
Cod	5	10	9·8	11	0·003	0·032	0·033	0·32
Sept. 1967		(12–31)	(3·5–19)	(3·2–20)	(0·027–0·11)	(0·004–0·068)	(0·012–0·057)	(0·23–0·44)
Salmon[a]	11	31	14	2·9	3·4	1·5	0·30	11·0
Autumn 1968		(20–53)	(7·7–20)	(1·1–8·2)	(0·26–7·1)	(0·005–3·1)	(0·014–0·54)	(1·2–20)
Fish oil	1	16	7·3	3·5				100
Oct. 1968								
Seal (grey) liver	1	90	41	44	3·9	1·7	1·8	4·1
Seal (common and grey)	2	130	62	30	66	32	15	52
Sept., Nov. 1968		(110–150)	(57–66)	(16–43)	(58–74)	(31–32)	(8·5–21)	(48–55)
Eggs from guillemot	9	570	20	250	40	1·2	16	7·0
May 1968		(300–700)	(7·5–38)	(140–360)	(20–51)	(0·7–2·3)	(7·9–21)	(3·6–11)
The Archipelago of Stockholm								
Mussel	15	3	1	5·2	0·04	0·02	0·037	1·1
Oct. 1966, Dec. 1967		(1–4·7)	(1–1·8)	(3·4–7·0)	(0·01–0·061)	(0·01–0·024)	(0·032–0·044)	(0·94–1·3)
Herring	4	7·7	3·9	6·1	0·23	0·11	0·17	2·6
May 1965		(4·3–11)	(2·0–5·3)	(3·3–8·5)	(0·094–0·30)	(0·044–0·15)	(0·073–0·23)	(2·2–2·8)
Seal (grey)[a]	3	170	17	30	36	4·2	6·1	27·1
May 1968		(97–310)	(11–21)	(16–56)	(35–36)	(2·4–6·6)	(5·7–6·4)	(11·5–37·5)
White tailed eagle								
March–June 1965–68	4	25,000		14,000	330		100	1·5
Pectoral muscle		(16,000–30,000)	n.d.	(8,400–17,000)	(200–400)	n.d.	(150–240)	(0·9–2·0)
Brain	3	1,800		910	100		47	5·4
		(1,700–2,100)	n.d.	(490–1,560)	(99–110)	n.d.	(29–70)	(4·6–6·0)
Eggs from white tailed eagle	5	1,000		540				5·6
May–June 1966		(610–1,600)	n.d.	(250–800)	n.e.	n.e.	n.e.	(3·4–9·1)
Heron								
April 1967	1	14,000	n.d.	9,400	71	n.d.	48	0·51
Gulf of Bothnia								
Herring	4	6·2	3·5	1·5	0·26	0·14	0·065	4·4
		(5·2–8·1)	(2·0–4·8)	(0·93–2·0)	(0·15–0·42)	(0·091–0·21)	(0·020–0·091)	(2·1–6·8)
Seal (ringed)	2	120	56	13	63	30	6·8	54
May–Oct. 1968		(110–130)	(54–57)	(9·7–16)	(58–68)	(28–31)	(5·0–8·5)	(52–55)
Gulf of Finland								
Seal pup (grey)	2	42	23	6·5	25	14	3·9	60
March 1968		(41–43)	(22–23)	(6·0–7·0)	(24–26)	(13–14)	(3·4–4·4)	(56–63)
Seal milk								
March 1968	1	36	21	4·5	11	6·5	1·4	31

[a] εDDT stands for DDT + DDE + DDD. For salmon and seal, there was respectively 41 per cent DDD in εDDT, the mean figure being 17 per cent.
n.e., Not estimated. n.d., Not detected.

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of Stockholm, stored in a non-heated room for six months, had a greater proportion of DDD (up to 40 per cent of ε DDT), and simultaneously smaller levels of DDT itself, than all other seals. This could have been caused by post mortem metabolism by microorganisms[16]. On the other hand, the ringed seal from the Gulf of Bothnia, stored in the same way as far as we know, had only about 5 per cent of DDD, but this low figure may be corrected with the uncertainty of DDD estimation at the time when the other seals were analysed. This difference between the archipelago seals and the other seals, however, could be related to the fact that the large amount of pollution and the land-water relation in the archipelago of Stockholm produce a higher microbiological activity in the water, transforming[17] DDT to DDD and possibly DDE.

Little is known about the toxicity of PCB in the levels found by us. PCB has, however, been proved to cause pathological changes in laboratory animals[18], and was found to be among the ten most potent chemicals among a hundred tested by injection in eggs[19]. Some teratogenic effects have been noted[19]. Effects of DDT, DDE and PCB on hepatic enzymes which increase the metabolism of progesterone, testosterone and oestradiol have also been published[6]. It is worth noting that bald eagles (*Haliaeetus leucocephalus*), fed a diet containing DDT, had between 58–86 p.p.m. DDT and DDD in their brains when they died[20]. Approximately the same values were found in robins and house-sparrows[21]. Brain tissue in white tailed eagles from the Stockholm area contained on average 100 p.p.m. (fresh tissue) of DDE. DDE, on the other hand, is less acutely toxic than DDT itself. The greatest value in North American bald eagles collected in the field in 1965 was 118 p.p.m. (mainly DDE) in muscle tissues[21]. The greatest level for sampled wild white tailed eagles from Sweden is 400 p.p.m. (mainly DDE) and the mean was 330 p.p.m. in muscle tissue (wet weight). Nothing is yet known about the PCB content in bald eagles from North America, but in eagles from our archipelago the mean value in muscle was 190 p.p.m. (brain 47 p.p.m.) and the greatest figure was 240 p.p.m. (brain 70 p.p.m.).

Neither the fresh tissue nor the original fat values were estimated in the addled eggs from white tailed eagles. Sometimes the water content was as low as 8 per cent (normally 70–75 per cent in fresh hen eggs). The values based on the fat content of these eggs are probably more reliable and we only report such figures. The levels, although high, were only 1/25 of those of the eagle fat. The residues of ε DDT and PCB in the guillemot eggs were about 50 per cent lower than those in the white tailed eagle eggs. The guillemots are vagrant in winter but most of the central Baltic population is believed to remain in the Baltic area the year round. It is not known whether the population is decreasing, but no drastic decline has been reported.

All species mentioned here (except mussel) are more or less vagrant, thereby giving a picture of the situation in a large water area. A relatively few analyses on a vagrant animal may not give a very accurate picture, but when different species are shown to contain more residues in the Baltic than in the North Sea and the Atlantic, the situation is more convincing.

In the Baltic proper, the Gulf of Bothnia and in the archipelago of Stockholm, the levels of chlorinated hydrocarbons are approximately ten times greater than the reported sparse figures for comparable species in the North Sea area and the Atlantic. This may be caused by several factors. The Baltic is surrounded by large areas of land and the water volume is comparatively small. Furthermore, the water exchange with the North Sea is very limited as a consequence of the thresholds (18 and 8 m deep) and the narrowness of the Danish sounds. Residues brought into the Baltic from the coasts and from air pollution will accumulate here to a great extent. This development is no doubt favoured by a generally low microbiological activity because of very low temperatures of the intermediate Baltic "winter water" during most of the year, and also of the deep water layers in the northern areas. As a consequence of the rather cold winter, this is also largely true for the upper surface layer.

The brackish water character of the Baltic also favours a concentration of residues in living organisms. The substances in question are probably more readily transported in a horizontal direction by living matter from fresh water to the low salinity surface water (frequently 5–6 per mille or less) of the archipelagos and along the Baltic coasts. In the North Sea, the discrepancy between the salt seawater and fresh water especially when combined with the effect of the tidal movements can result in a faster decomposition of freshwater organisms and of organic matter, including the chlorinated hydrocarbons. The increased and more immediate contact here with the microbiological activity located in the bottom material may be important. Furthermore, there is in the Baltic proper a pronounced stratification of the water. A distinct halocline delimits the deep water of higher salinity upwards (usually in 50–70 m depth). In the warm season, the uppermost surface layer is separated from the "winter water" by a very marked thermocline (usually in 15–20 m depth, sometimes supported by a secondary halocline). As a result of these two discontinuity layers and, especially in coastal areas, of the absence of marked tidal currents, the vertical water exchange and movements are more or less blocked. The contact of sinking organic matter with the bottom material is thus to a great extent delayed. In the discontinuity layers of the Baltic Sea, living and dead plankton and other organic matter are concentrated, as a consequence of differences in the specific gravity of the water. The layers mentioned are important feeding areas for plankton and pelagic fish such as herring and sprat. In the Sound, in the archipelago of Stockholm and in the Idefjord, local sources of pollution can be important as the residue levels indicate.

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Toxicity and distribution of Aroclor® 1254 in the pink shrimp *Penaeus duorarum**

D. R. Nimmo, R. R. Blackman, A. J. Wilson, Jr. and J. Forester

Environmental Protection Agency, Gulf Breeze Laboratory; Sabine Island, Gulf Breeze, Florida, USA

Abstract

The polychlorinated biphenyl Aroclor® 1254 was released in an accidental leakage of heat-exchange fluid from an industrial plant, into the Escambia River, near Pensacola, Florida, USA. This material was carried downstream, and is now found in the fauna of Escambia Bay and its contiguous waters, prime nursery areas for fishes and invertebrates such as penaeid shrimp. The significance of pollution by this chemical was assessed by establishing toxicity levels, determining routes of entry, and investigating its movement and distribution in various tissues of shrimp under controlled conditions in the laboratory. Aroclor 1254 added to the water was toxic to the juvenile pink shrimp *Penaeus duorarum* at a concentration of 1.0 part per billion within 15 days, but was less toxic to adult pink shrimp. Shrimp obtained the contaminant from water and food and concentrated it to 510.0 parts per million in the hepatopancreas. Aroclor 1254 residue data from shrimp collected in the estuary are included in the study.

Introduction

Since 1966, pesticide toxicologists and ecologists have become increasingly aware of polychlorinated biphenyls (PCBs). First discovered in fishes, feathers, and human hair (Jensen, 1966), residues have since been found in many organisms from diverse areas of the world. Structurally, PCBs resemble chlorinated hydrocarbon pesticides such as DDT, and are widely used in formulating plastics, resins for rubber-based lacquers, varnishes, paints, lubricants, heat-transfer fluids and electrical insulators. PCBs are relatively

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* Contribution No. 128, Gulf Breeze Laboratory.

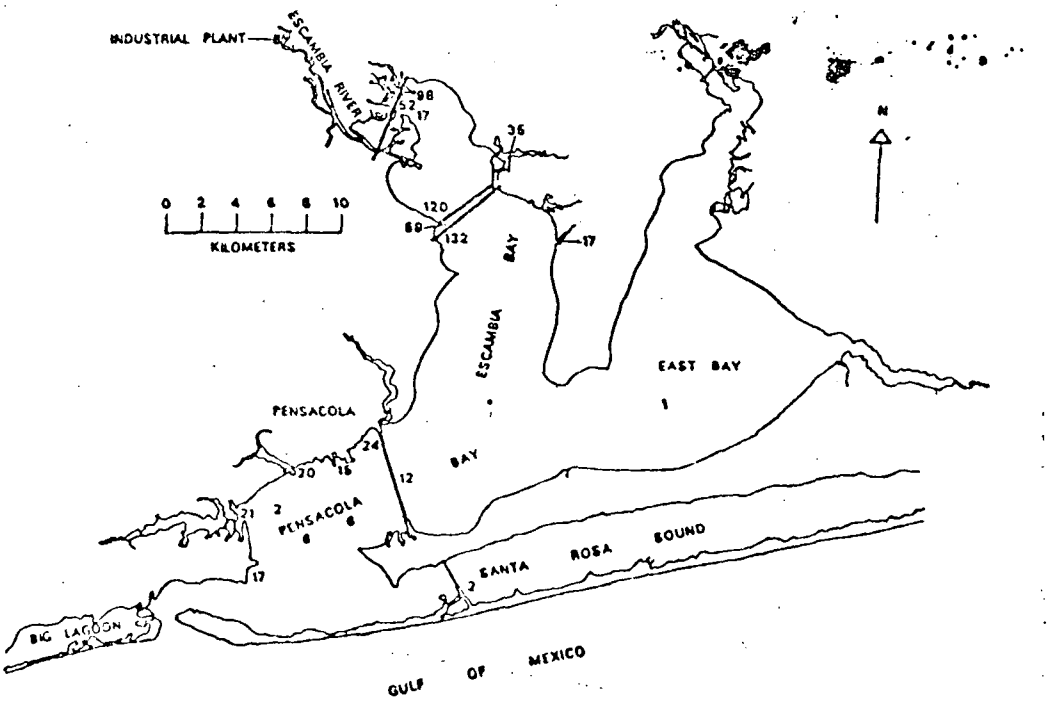

Fig. 1. Residues of Aroclor 1254 (in ppm) found in shrimp hepatopancreases from Escambia Bay and contiguous waters during 1969/1970. Each sample listed represents composite tissues of at least 5 individuals

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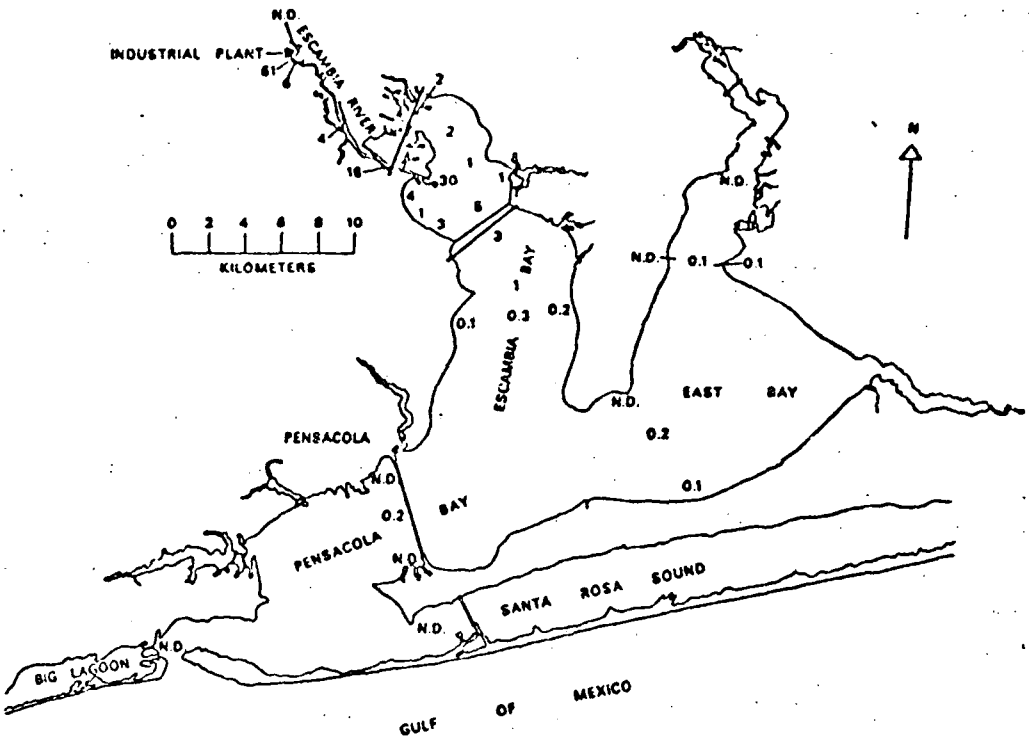

Fig. 2. Residues of Aroclor 1254 (in ppm) found in sediments from Escambia Bay and contiguous waters during 1969/1970. N.D.: less than 0.03 ppm

insoluble in water, but soluble in lipid and lipid solvents. In addition to their thermal stability they are also resistant to acid and base, and therefore, persist in the environment.

PCBs are toxic to trout and blue gill (spp. not given) — GUSTAFSON, 1970; shrimp (*Penaeus duorarum*) and oysters (*Crassostrea virginica*) — DUKE et al., 1970; and a fish (*Lagodon rhomboides*) — HANSEN et al., 1971. Also, a PCB used as a binder in epoxy paint was toxic to chickens (GUSTAFSON, 1970). Abnormally thin-shelled eggs of birds in Great Britain and North America were associated with residues of chlorinated hydrocarbons including the PCBs (RISEBROUGH et al., 1968). In 1969, a PCB (Aroclor 1254) was discovered as a contaminant in water, sediment and fauna of Escambia Bay, Florida (DUKE et al., 1970). One source of this material was traced to an accidental leak in a heat-exchange system of an industrial plant located several kilometers upstream in Escambia River. It is now present in estuarine organisms, including shrimp captured from Escambia Bay and contiguous waters (Fig. 1). Sediments from the river and upper bay appear to be a reservoir for the compound (Fig. 2). In earlier experiments, shrimp exposed to these sediments for 30 days accumulated the chemical (NIMMO et al., 1971). In this paper we report toxicity of Aroclor 1254 in water, rates of accumulation from food and water, and distribution of this PCB in the organs of the pink shrimp *Penaeus duorarum*.

Materials and methods

Shrimp for laboratory studies were obtained from two sources. Juvenile (2.5 to 3.8 cm) pink shrimp (*Penaeus duorarum*) were collected with a small net from Santa Rosa Sound at Pensacola Beach, Florida, in June through September. Adult pink shrimp from Tampa, Florida, were purchased from a live-bait dealer. Background concentrations of chlorinated hydrocarbon compounds in the hepatopancreases of all shrimp never exceeded 0.6 parts per million (ppm); whole-body residues were less than 0.01 ppm.

All shrimp were acclimated in flowing sea water for several days in the laboratory. Juveniles fed on detritus carried in by the flowing unfiltered sea water and adults were fed mullet (*Mugil cephalus*) muscle containing less than 0.03 ppm organochlorine compounds each day. Beach sand with no detectable organochlorine compounds was provided as a substrate for the shrimp.

Shrimp were exposed to Aroclor 1254 (hereafter called Aroclor) in flowing-water systems. The Aroclor was dissolved in polyethylene glycol 200, infused into the flowing water with syringe pumps, then mixed by

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a series of baffles as it flowed into aquaria. The volume of the aquaria varied from 18 to 160 l commensurate with the numbers and sizes of test animals and flow-rate of water. Animal to volume ratio was 1 animal per 2 l water. Concentrations of Aroclor were routinely measured by gas chromatography. No attempt was made to control salinities which ranged from 25 to 32‰, but electric aquarium heaters were used to maintain water temperatures between 20° and 30 °C.

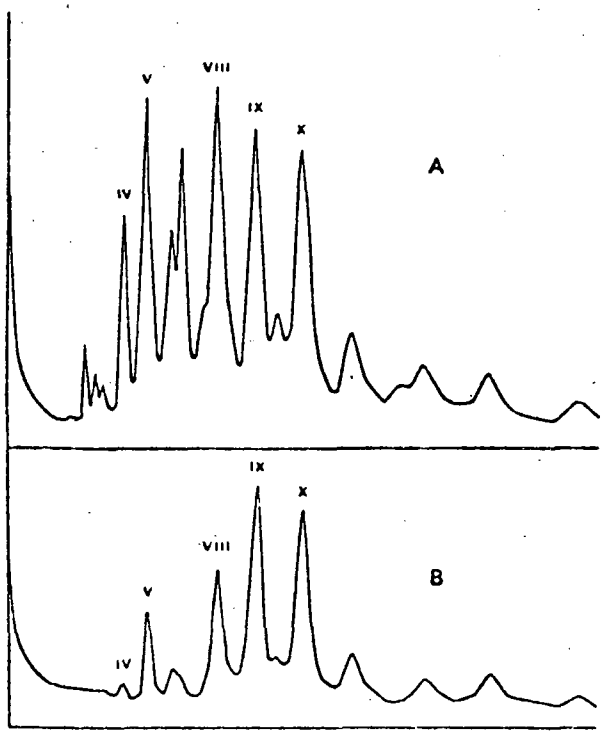

Fig. 3. Aroclor chromatograms. (A) Aroclor 1254 standard, (B) Aroclor 1254, recovered from *Penaeus duorarum* hepatopancreas after shrimps had been fed fish containing material (see Table 4). Concentrations of DDT and its metabolites were negligible, therefore, no attempt was made to separate them from Aroclor isomers. Gas flow 25 ml/min, nitrogen; injection temperature 210 °C, oven temperature 190 °C, detector temperature 210 °C; H^3 electron capture detector; 152.4 × 0.317 cm glass-column packed with 2% OV-1 on 100/120 Gas Chrom Q

Concentrations of Aroclor in shrimp were determined from pooled samples by gas chromatography. When a group of 10 shrimp was exposed to a constant concentration of Aroclor in flowing-water, individual residues differed by a factor of 10. Consequently, laboratory analyses are from composite samples of at least 10 individuals, unless stated otherwise. All analyses on *Penaeus duorarum* from Escambia Bay and contiguous waters were on composite samples of at least 5 individuals.

Depending on type and weight of the samples, four methods of preparation were employed. (1) Samples of shrimp or food items larger than 1 g were mixed with anhydrous sodium sulfate in a blender and extracted for 4 h with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and eluted from a Florisil column with 6% ethyl ether in petroleum ether. (2) Samples less than 1 g were analyzed by a modification of the micro-method of ENOS (private communication). Samples were weighed in Duall[1] tissue grinders and extracted with three 2.0 ml portions of acetonitrile. The acetonitrile extracts were combined and diluted with 6 ml of 2% Na_2SO_4 in distilled water, then agitated and extracted with three 2.0 ml portions of hexane. These extracts were combined and concentrated to about 0.5 ml, then transferred to a Size "B" Chromaflex[1] column containing 1.5 g of Florisil topped with 1.5 g of anhydrous sodium sulfate. The residue was eluted from the column with 20.0 ml of 1% ethyl ether in hexane. (3) Water samples were extracted with petroleum ether, then the extracts were dried with anhydrous sodium sulfate and reduced to an appropriate volume. (4) Sediments were analyzed by the method of NIMMO et al. (1971).

All eluates were adjusted to an appropriate volume for analysis by electron-capture gas chromatographs equipped with OV-1 columns. Quantitation of Aroclor 1254, a multiple-peaked compound, was made by averaging the heights of 5 major peaks which had retention times relative to aldrin of 1.31 (*IV*), 1.55 (*V*), 2.32 (*VIII*), 2.74 (*IX*) and 3.27 (*X*) (Fig. 3). Interference from DDT was negligible due to the relatively high residues of Aroclor 1254 in most samples. Laboratory tests indicated recovery rates above 80%, but data in this report do not include a correction factor for recovery. The presence of Aroclor 1254 in shrimp and sediments was verified by mass spectroscopy at the Environmental Protection Agency Laboratory, Athens, Georgia.

Results

Acute and chronic biosassays

Acute toxicity tests at this laboratory showed that Aroclor was about one tenth as toxic to juvenile *Penaeus duorarum* as DDT (Table 1). For example, 10.0 parts per billion (ppb) DDT in the water killed 100% of a population of shrimp in 96 h, whereas 100.0 ppb in the water was necessary to obtain the same results with Aroclor.

In chronic flowing-water bioassays, Aroclor at 0.94 ppb killed 51% of the juvenile shrimp (2.5 to 3.8 cm) within 15 days (Table 2). Juvenile shrimp were more sensitive to Aroclor than adults. Exposure to 3.5 ppb for 35 days resulted in a mortality of 50% in a group of adult shrimp (9.5 to 12.5 cm). The data

[1] Kontes Glass Co., Vineland, N. J., USA.

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show the need for challenging several stages in the life cycle of shrimp with chemicals such as Aroclor.

We have observed that symptoms of Aroclor poisoning in shrimp are different from those of most organochlorine insecticides. Pink shrimp which we exposed to 0.15 ppb or more DDT showed nervous impairments such as tremors, loss of equilibrium and, finally, paralysis as defined by cessation of locomotor movements. In tests with Aroclor, regardless of its concentration, shrimp showed delayed mortality and died at a rate of one or two per day with no apparent prior symptoms of poisoning. Like others (Duke, et al., 1970; Wildish, 1970), we suggest that crustaceans may be more susceptible to the chemical during molting.

Table 1. *Penaeus duorarum. Comparison of toxicities of p, p' – DDT and Aroclor 1254 to shrimp in flowing-water tests. Mean temperature and salinity of sea water in DDT experiment were 24 °C and 28 ‰, respectively; for Aroclor 1254, 19 °C and 31 ‰*

DDT[a]			Aroclor 1254[b]		
Test concentration (ppb)	Mortality 48 h (%)	96 h (%)	Test concentration (ppb)	Mortality 48 h (%)	96 h (%)
10.0	100	100	100.0	80	100
1.0	30	80	10.0	0	0
0.5	0	40	1.0	0	0
0.1	10	20	Control	0	0
Control	0	0			

[a] Personal communication, J. I. Lowe, Environmental Protection Agency, Gulf Breeze, Florida 32561, USA.
[b] Duke et al., 1970.

Accumulation and transfer of Aroclor in tissues

The uptake of Aroclor from water by adult *Penaeus duorarum* (3.8 to 7.6 cm) and the translocation to the hepatopancreas, whole body and abdominal muscle was measured (Fig. 4). Accumulation was linear with time in the hepatopancreas ($r = 0.97$) and whole body ($r = 0.90$), but a plateau was reached

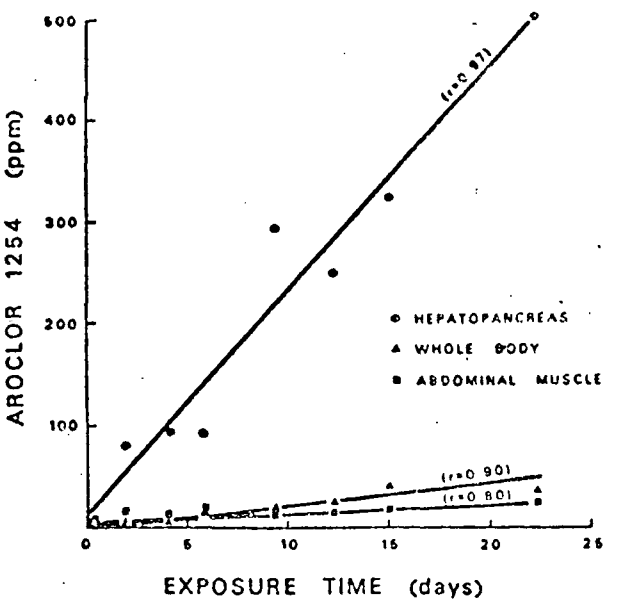

Fig. 4. *Penaeus duorarum.* Rates of absorption of Aroclor 1254, in various tissues of shrimp exposed to 2.5 ppb of the chemical in flowing water. Unexposed shrimp showed no detectable residue. r = correlation coefficient

Table 2. *Results of chronic bioassays with Aroclor 1254 and the pink shrimp Penaeus duorarum in flowing water*

Shrimp rostrum-telson length (cm)	Concentration[a] (ppb)	Average salinity (‰)	Average temperature (°C)	No. of test individuals	Replicates	Days exposed	Average mortality (%)	Level of significance
2.5 – 3.8	Control	32	29	65	5	15	12	—
2.5 – 3.8	0.57	32	29	20	2	15	30	0.10[b]
2.5 – 3.8	0.94	32	29	45	3	15	51	0.005[b]
2.5 – 3.8	9.4	32	29	20	2	15	90	0.001[b]
2.5 – 3.8	19.0	32	29	20	2	15	100	0.001[b]
4.2 – 7.2	Control	29	28	25	1	32	4	—
4.2 – 7.2	2.4	29	28	20	1	17	65	0.001[c]
4.2 – 7.2	3.1	29	28	25	1	32	80	0.001[c]
6.6 – 9.0	Control	29	20	43	1	53	26	—
6.6 – 9.0	4.3	29	20	46	1	53	83	0.001[c]
7.6 – 8.5	Control	31	29	60	1	18	9	—
7.6 – 8.5	4.0	31	29	60	1	18	41	0.001[c]
9.5 – 12.5	Control	28	20	50	1	35	8	—
9.5 – 12.5	3.5	28	20	50	1	35	50	0.001[c]

[a] Average of at least three determinations.
[b] Student's *t*-test.
[c] Chi-square.

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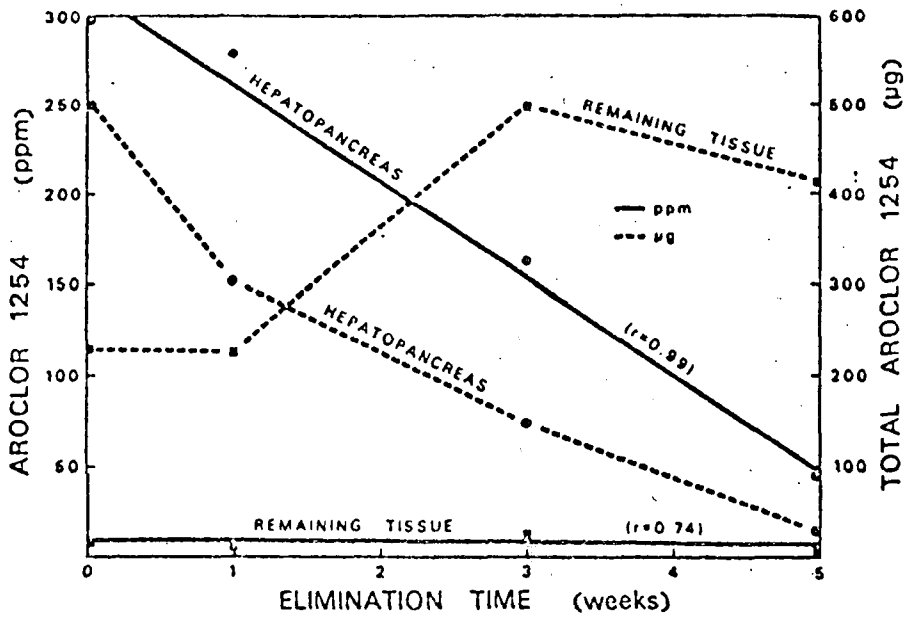

Fig. 5. *Penaeus duorarum*. Rates of elimination from hepatopancreas and subsequent increase in remaining tissue. Shrimp were exposed to 7.5 ppb Aroclor 1254 in flowing water for 16 days, then placed in Aroclor-free environment. Composite tissue samples from 8 individuals were analyzed for each determination. Unexposed shrimp showed no detectable residues. r = correlation coefficient

in the muscle within 2 days, with little increase thereafter. Residues in the hepatopancreas reached 510.0 ppm after 22 days and represented a 2.04×10^5 increase over the 2.5 ppb Aroclor in the test water. During this experiment, 50% of the exposed and 7% of the unexposed shrimp died.

In a subsequent experiment, most of the Aroclor was lost from the hepatopancreas and transferred to other tissues (Fig. 5). Adult shrimp (9.5 to 11.5 cm) were first exposed to 7.5 ppb Aroclor in the water for 16 days, then placed in an Aroclor-free environment for 5 weeks. Total weight (µg) in the hepatopancreas decreased by 80% in 5 weeks, but that in the remaining tissues almost doubled. Whole-body loss was from 731 to 466 µg or about 60% in 5 weeks.

A slightly different picture exists concerning the loss of Aroclor from the tissues if we express the amount in parts per million (Fig. 5). Aroclor (in ppm) showed little change in tissues other than the hepatopancreas during post exposure. In contrast, the rate of elimination from the hepatopancreas was constant and linear with time ($r = 0.99$), the biological halflife in this organ being 17 days. Aroclor is more persistent in the other tissues of shrimp than DDT, the insecticide being completely eliminated in 3 weeks (NIMMO et al., 1970). While the *Penaeus duorarum* were held in the Aroclor-free environment, 23% of the exposed shrimp died, with no loss of the unexposed.

Accumulation in body organs

Residues found in laboratory experiments are compared with those in natural populations of shrimps in Escambia and Pensacola Bays in Table 3. In all tests, the shrimp incorporated the chemical. The proportion of Aroclor in tissues of shrimp which were exposed to 0.2 ppb in the water for 50 days was nearest to that found in shrimps captured alive from the bays.

The distribution of Aroclor in the tissues of shrimp is much the same as DDT, maximum amounts occurring in the hepatopancreas and least in abdominal muscle or exoskeleton (NIMMO et al., 1970). Generally, the distribution of Aroclor in the tissues of *Penaeus duorarum* corresponded to the amount of lipid in the tissues.

We believe water and food are sources of Aroclor to shrimp, but we do not know which contributes more. FARFANTE (1969) summarized earlier work on the feeding habits of shrimp and reported that the three commercially-important penaeid shrimps, pink *Penaeus duorarum*, white *P. setiferus*, and brown *P. aztecus*, are omnivorous. Some of the contents found in digestive tracts by other investigators include inorganic debris, detritus, and a variety of algae, including diatoms. Aroclor attached to detrital material in aquaria or in field substrates was probably ingested by the shrimp.

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Table 3. *Distribution of Aroclor 1254 in tissues of exposed Penaeus duorarum which had accumulated chemical from water and food and in penacid shrimps from natural populations in the Pensacola estuary, Florida, USA*

Methods of exposure	Hepato-pancreas ppm	Ventral nerve	Digestive tract	Heart	Gills	Exo-skeleton	Abdominal muscle
Water							
Pink shrimp exposed to 3.5 ppb Aroclor in water for 35 days	108	120	32	77	59	14	15
Pink shrimp exposed to 0.2 ppb Aroclor in water for 50 days	30	3.3	3.2	2.8	1.4	0.8	0.7
Food							
Pink shrimp fed spot[a] (43.0 ppm whole body) for 16 days	146	39	15	25	38	6.5	57
Pink shrimp fed spot (field-captured, 0.2 ppm whole body) for 16 days	0.6	0.5	<0.1	0.3	0.3	0.2	<0.1
Pink shrimp fed croaker[b] (0.68 ppm in muscle) for 50 days	5.8	2.0	1.0	1.3	0.9	0.6	0.6
Natural populations							
Pink shrimp captured 19. I. 1970[c]	15	9.4	1.1	—	1.4	0.6	0.9
Pink shrimp captured 3. IV. 1970[c]	4.6	0.6	0.5	0.5	0.3	0.2	0.1
White shrimp captured 28. VIII. 1970[d]	17	4.0	1.5	1.0	0.8	0.8	1.3
Brown shrimp captured 28. VIII. 1970[d]	98	14	9.7	4.2	3.1	2.2	0.9

[a] The spot *Leiostomus xanthurus* were previously exposed to 5.0 ppb Aroclor 1254 in the water for 12 days.
[b] Atlantic croaker *Micropogon undulatus* were captured in Escambia Bay, Florida.
[c] Pensacola Bay.
[d] Escambia Bay.

Table 4. *Percentages[a] of 5 peaks in Aroclor 1254 recovered from water, fish and Penaeus duorarum*

Item	Peak (%)				
	IV	*V*	*VIII*	*IX*	*X*
Standard	10.0	18.5	25.0	22.2	20.0
Water (30 ‰ S)	8.2	15.6	30.0	22.8	23.1
Fish muscle[b]	5.1	10.8	27.4	27.9	28.4
Shrimp hepatopancreas[c]	2.8	10.9	23.9	31.1	31.1

[a] $\frac{\text{peak height}}{\text{sum of 5 peaks}} \times 100$.
[b] The Atlantic croaker *Micropogon undulatus* were captured from Escambia Bay and contained 0.68 ppm Aroclor 1254.
[c] The shrimp *Penaeus duorarum* were fed Atlantic croaker muscle.

We investigated differences in the proportion of Aroclor peaks with respect to the standard; the change is greatest in the hepatopancreas of shrimp (Fig. 3; Table 4). Heights of 5 peaks used for quantitation of the chemical show the greatest reduction in peaks *IV* and *V*, with some increase in *IX* and *X*. We do not know whether this reflects actual alterations in the molecules or differential solubilities in the various systems.

Results of field studies

Distribution of shrimp in the estuary in relation to salinity is a factor which regulates the amount of Aroclor in the body (Fig. 1). The brown shrimp *Penaeus aztecus* from upper Escambia Bay had the highest residues (132.0 ppm in the hepatopancreas). The white shrimp *P. setiferus* from the mouths of small streams emptying into Escambia Bay had a maximum residue of 59.0 ppm. The highest residue in the pink shrimp *P. duorarum* captured in Pensacola Bay was 15.0 ppm. *P. setiferus* is most abundant in low salinity waters of less than 10‰ and *P. aztecus* occurs mostly in waters of 10‰ or more; the abundance of *P. duorarum* is not as dependent on salinity (Farfante, 1969). Although this distribution may vary with locale, types of substrate and seasonal temperatures, shrimp with the highest residues in this study were those captured in the lower salinities. Because higher concentrations of Aroclor occur in the sediments of upper Escambia Bay (Fig. 2), it is possible that burrowing activities of brown shrimp in these sediments could have caused additional absorption of leached chemical through the gills (Nimmo et al., 1971) as well as ingestion of contaminated food.

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Discussion and conclusions

In our investigations, there has been no evidence that Aroclor in the water, sediments, or biota in Escambia Bay was toxic to shrimp. We found no dead or dying shrimp in areas of "fish kills" which occurred frequently during summer months of the past two years. Aroclor in water samples collected 48 cm above the sediments in the upper Bay was below that considered toxic to shrimp, but it was detectable (0.06 ppb in unfiltered water). It probably leached from the sediments or was attached to suspended particulate matter. Adult shrimp in live cages placed directly on sediments in upper Escambia Bay for 3 weeks did not die nor did the "controls" held 48 cm above in an uncontaminated substratum. Shrimp on lower sediments accumulated almost 3 times more Aroclor (6.7 ppm in the hepatopancreas) than did controls.

Nevertheless, our laboratory investigations show that Aroclor 1254 in solution is toxic in the 1 ppb range to shrimp. Therefore, the occurrence of this chemical in the water of Escambia Bay or in other estuarine areas is reason for concern. We also believe the residues found in shrimp from the Escambia Bay are high enough to be of importance, although we have not found a correlation between residues and mortality. If postlarval or juvenile shrimp were exposed directly to the sediments in upper Escambia Bay for a period of weeks, a threat could exist because of availability of PCB-laden detritus and also the leaching of the chemical at the water-substrate interface. We are now investigating this possibility.

Deaths of shrimp due to this or any other contaminant in natural environments would be difficult to observe. Except as larvae, shrimp are primarily benthic, secretive animals, and hide by burrowing in the sediment. If they are active at night, they usually remain below the substrate by day. Here, they may obtain higher concentrations of Aroclor than when swimming, and if they die, dead shrimp do not surface after dying as do fish, rather they decompose rapidly or are quickly eaten by predators.

Aroclor occurs in the tissues of shrimp which were captured several kilometers from the original sources of the material in Escambia Bay (Fig. 1). This suggests the potential of this material to be dispersed through the ecosystem, and it might be available to man through his seafood. We believe this contamination should serve as a warning to increase monitoring of the environment for Aroclor and related industrial materials.

Summary

1. A concentration of 1.0 part per billion of Aroclor in the water kills the juvenile pink shrimp *Penaeus duorarum* within 15 days in the laboratory. Adult shrimp are not as susceptible, but higher concentrations of 2.4 to 4.3 ppb kill within 17 to 53 days — depending on the maturity of test individuals.

2. In laboratory tests in which Aroclor was added to the water, *P. duorarum* absorbed the material and concentrated it in the hepatopancreas. Subsequently, it was transferred to other tissues, then lost. It was more persistent in the tissues of shrimp than the chemically-related pesticide, DDT.

3. Aroclor was absorbed from the water and its subsequent distribution in tissues was similar to that found in wild shrimp from contaminated areas. In the laboratory, shrimp also obtained the Aroclor from food, and we conclude that both sources are available to shrimp in the Pensacola estuary.

4. Concentrations of Aroclor in *P. duorarum* from the estuary appear to reflect distribution patterns in shrimp. Aroclor in shrimp captured at considerable distances (up to 24 km) from the original source demonstrate the potential for dispersion and concentration of this material in an ecosystem.

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First author's address: Dr. D. R. Nimmo
Environmental Protection Agency
Gulf Breeze Laboratory
Sabine Island
Gulf Breeze, Florida 32561
USA

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