E. P. Wheeler

PCB Residues in Atlantic Zooplankton

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Studies of the polychlorinated biphenyls in marine ecosystems that have been accomplished to date have shown that these compounds are more abundant than the chlorinated hydrocarbons of agricultural origin in the majority of ecosystems examined. Thus, PCB was present in higher concentrations than the DDT compounds in marine birds of the south Atlantic (1), in fish from the north Atlantic (2), in the Brown Pelican (Pelecanus occidentalis) populations of Florida (3), in fish and fish-eating birds from Long Island Sound (4), in marine birds of Amchitka Island in the northern Pacific (5), in some of the marine birds from Peru (6), and in sea birds breeding in Antarctica but spending the remainder of the year in Australian waters (1). Apparently only in the waters of coastal California and northwestern Mexico (7, 8) and in the Baltic (9) are DDT compounds more abundant than PCB. In California the exceptionally high concentrations of DDT compounds in marine organisms are associated with the large amounts of DDT residues in the effluent of a DDT manufacturing company (10, 11).

Most of the accumulated data refer to fish and fish-eating or tube-nosed marine birds. In this paper we present the results of measurements of PCB and other chlorinated hydrocarbons in zooplankton samples from the Atlantic Ocean. A traditional dogma of pollution ecology has asserted that residues of non-polar chlorinated hydrocarbon pollutants such as PCB are trophically accumulated in food chains. Ample documentation is available to support this thesis in terrestrial food chains or in food webs that pass from fish or other aquatic organisms to mammals or birds, but the data that might support the food chain concentration theory in marine and fresh-water food webs consisting only of fish and invertebrates are surprisingly few.

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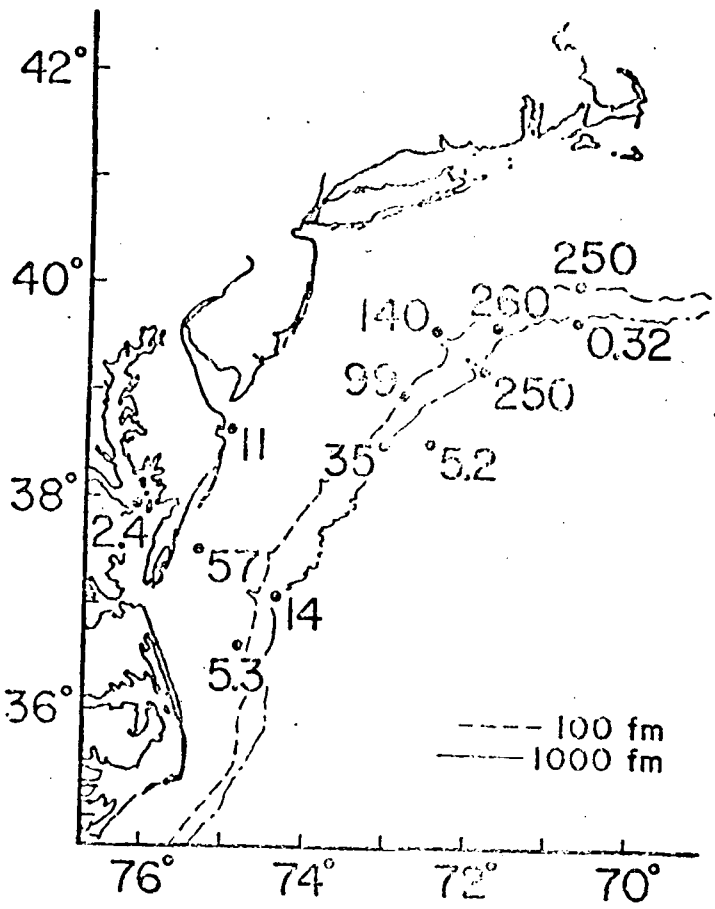

Figure 1: PCB concentrations in zooplankton of the north-west Atlantic Shelf, parts per million of the lipid weight

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MATERIALS AND METHODS

Samples were obtained during three cruises of R/V ATLANTIS II of the Woods Hole Oceanographic Institution with a #6 mesh plankton net. Station sites and other relevant data are presented in Table I. The plankton were immediately transferred to polycarbonate or glass jars and frozen. Collection of marine organisms from oceanographic vessels for pollutant analysis requires that precautions be taken to prevent accidental contamination during collection and storage (12). Hexane rinses of the collection jars, followed by concentration and gas chromatographic analysis, showed that they contained no PCB. Chips of the marine paint used on ATLANTIS II were also extracted with hexane and found to be free of PCB. Since PCB has in the past been added to hydraulic fluids to reduce flammability, contamination from leakage of hydraulic fluids on the vessel was another potential source of the PCB found. No halogenated biphenyls have been used, however, in the recent past as components of hydraulic fluids on WHOI vessels. Chlorinated paraffins were added in 1969 to reduce flammability, but this use was discontinued in 1970 (Mobil Service Dept., personal communication). Hexane extracts of the mesh of new plankton nets were free of PCB, but PCB with chlorine composition similar to that of Aroclor 1254 could be extracted from nets that had been in use. We conclude that the source of this PCB was the water, with associated organisms and petroleum droplets, through which the nets had been towed. A fraction of the PCB in the plankton may therefore be secondarily derived from the net. Relatively high PCB concentrations in fish livers from the same localities indicate the presence of PCB in the ecosystems examined (2).

Plankton samples obtained on ATLANTIS II cruise 52 by J. H. Ryther were analyzed in Berkeley. They were placed in a 65° oven overnight for determination of dry weight, ground with anhydrous sodium sulfate, and Soxhlet-extracted with 2:1 hexane:acetone. All materials extracted by this procedure were defined to be lipids, although various amounts of carotenoid pigments were also present. Because the latter compounds are comparatively non-polar, it is likely that PCB dissolves readily in them. The comparative efficiencies of extraction with hexane alone and with 2:1 hexane:acetone were not determined. Lipid samples were dried overnight at 65° for lipid weight determinations.

Cleanup was accomplished by passage through a celite: sulfuric acid:fuming sulfuric acid column (13) or by shaking lipid extracts in petroleum ether in a 500 ml erlenmeyer flask with 2 volumes of fuming sulfuric acid. The flask was placed on dry ice, causing the acid mixture to freeze, permitting the removal of the liquid petroleum ether fraction (14). Aliquots

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TABLE I

PCB and DDT residues in zooplankton. Parts per million wet weight (W), dry weight (D), or lipid weight (L).

Station, Sample Number, Date	Position	Depth (meters) Time Out	p,p'-DDT	p,p'-DDE	PCB	PCB/Total DDT
North-west Atlantic Shelf						
Asterias 12-71 12/10/71	41°30'N 70°40'W	0-12 30 min	N.D.	N.D.	23 (L)	----
A-II-52-1498 9/7/69	39°43'N 70°40'W	0-30 15 min	0.007 (L) 0.0015 (D)	0.035 (L) 0.010 (D)	0.32 (L) 0.071 (D)	7.7
A-II-52-1499 9/7/69	40°04'N 70°40'W	0-25 7 min	N.D.	7.0 (L)	250 (L)	36
A-II-52-1512 9/11/69	39°37'N 71°40'W	0-10 10 min	N.D.	N.D.	260 (L)	>50
A-II-52-1513 9/12/69	39°13'N 71°50'W	0-10 15 min	N.D.	N.D.	250 (L)	>50
A-II-52-1514 9/12/69	39°37'N 72°25'W	0-10 13 min	N.D.	N.D.	144 (L) 1.9 (D)	>50
A-II-52-1520 9/14/69	38°59'N 72°50'W	0-85 13 min	N.D.	N.D.	99 (L) 3.0 (D)	>50

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TABLE I
(Continued)

Station, Sample Number, Date	Position	Depth (meters) Time Out	p,p'-DDT	p,p'-DDE	PCB	PCB/Total DDT
North-west Atlantic Shelf (continued)						
A-II-52-1521 9/14/69	38°32'N 72°25'W	1800 1 hr 38 min	0.08 (L)	0.21 (L)	5.2 (L)	18
A-II-52-1522 9/16/69	38°33'N 73°00'W	0-20 15 min	N.D.	0.54 (L) 0.016 (D)	30 (L) 0.87 (D)	55
A-II-52-1525 9/17/69	38°39'N 74°59'W	0-20 11 min	N.D.	2.4 (L) 0.11 (D)	11 (L) 0.53 (D)	4.8
A-II-52-1527 9/17/69	37°30'N 75°21'W	0-20 13 min	N.D.	N.D.	57 (L)	>50
A-II-52-1532 9/20/69	37°56'N 76°10'W	Surface 10 min	N.D.	N.D.	2.4 (L) 0.10 (D)	----
A-II-52-1539 9/23/69	36°35'N 74°51'W	0-20 13 min	N.D.	0.090 (L) 0.003 (D)	5.3 (L) 0.18 (D)	60
A-II-52-1541 9/23/69	37°04'N 74°25'W	0-20 20 min	N.D.	0.34 (L) 0.015 (D)	13.6 (L) 0.65 (D)	40
Open North Atlantic						
A-II-59-22 11/70	23°41'N 34°29'W	0-100	<0.00001 (W)	<0.00001 (W)	0.30 (W)	>30,000

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TABLE I
(Continued)

Station, Sample Number, Date	Position	Depth (meters) Time Out	p,p'-DDT	p,p'-DDE	PCB	PCB/Total DDT
Open North Atlantic (continued)						
A-II59-36 12/70	30°52'N 47°30'W	0-100 60 min	<0.00001 (W)	N.D.	0.45 (W)	>45,000
Open South Atlantic						
A-II-60-4-15 4/71	34°05'S 42°40'W	0-200 20 min	0.50 (L) 0.003 (W)	N.D.	120 (L) 0.64 (W)	200
A-II-60-4-33 4/71	32°30'S 26°24'W	0-200 20 min	0.61 (L) 0.001 (W)	N.D.	53 (L) 0.12 (W)	100
A-II-60-5-PT-106 6/71	14°03'S 05°55'E	0-200 20 min	0.068 (L) 0.0002 (W)	0.023 (L) 0.00007 (W)	7.3 (L) 0.021 (W)	80
A-II-60-5-PT-89 5/71	32°49'S 13°09'E	0-200 20 min	0.132 (L) 0.0002 (W)	N.D.	10 (L) 0.018 (W)	80

N.D.: Not Detected

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Polychlorinated Biphenyls and Organochlorine Pesticides in Some Freshwater and Marine Fishes

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Polychlorinated biphenyls (PCB) were found in higher concentrations than organochlorine pesticides in all fish analysed. Concentrations of PCB in herring *(Clupea harengus)*, salmon *(Salmo salar)*, and mussel *(Mytilus edulis)* were higher than those reported by Jensen et al. for the same species from Swedish coastal waters (1). Concentrations of PCB in cod *(Gadus morhua)* were approximately the same in both cases. No comparative data for eel *(Anguilla rostrata)* and pickerel *(Esox niger)* are available. PCB concentrations in pike *(Esox lucius)* were reported from The Netherlands and Sweden (2).

Levels of DDT and metabolites are similar to those found by Jensen et al. (1) and lower than reported recently for Canadian Atlantic fishes (3). Highest levels of DDT and metabolites in salmon are reported from Sweden (1) and the lowest from Canada (3).

EXPERIMENTAL

Eel and pickerel were taken from several lakes and from the Saint John River system in New Brunswick, Canada, in summer 1970. Groundfish (cod *(Gadus morhua)*, hake *(Urophycis tenuis)*, plaice *(Hippoglossoides platessoides)*, and ocean perch *(Sebastes marinus)*) were taken on Nova Scotia banks in summer 1970. Mackerel *(Scomber scombrus)* and one sample of herring were taken in the Bay of Fundy in September and December 1970, respectively. Two samples of herring were taken in Chedabucto Bay, Nova Scotia, in January 1971; mussels were obtained from Miramichi Bay, New Brunswick, in October 1970. Salmon (grilse) were taken in the Northwest Miramichi River, New Brunswick, in June 1970. The samples were kept frozen until analysis. Whole fishes and mussels with shells were weighed before analysis. Whole herring, shucked mussels, the muscle of all other fishes, and samples of eel and pickerel liver were analysed. Two to ten specimens were usually combined and analysed in duplicate as one group.

Samples (5-6 g) were ground with anhydrous sodium sulfate and extracted with hexane in Soxhlet extractors for 1 h. The thimbles were pre-extracted with hexane. Volume of the extract was adjusted to 100 ml and an aliquot (5-25 ml) was evaporated in vacuum on a rotatory evaporator to approximately 1.5 ml and applied to

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a column (45 x 0.7 cm) containing 2 g of alumina (for chromatography, Fisher Scientific Co.), pretreated as described (4). The column was washed with hexane, the effluent (20 ml) was concentrated to 1.5 ml and applied to a column (45 x 0.7 cm), containing silicic acid (Mallinckrodt SILICAR®, 100-200 mesh), pretreated as described (5). The column was washed with hexane, collecting 10 ml (fraction I) and 20 ml (fraction II) of the effluent, and with 10 ml of 10% ether in hexane (fraction III). The fractions were evaporated just to dryness in a rotatory evaporator, dissolved in a known volume of hexane (0.2-1.0 ml) and analysed by gas chromatography (6).

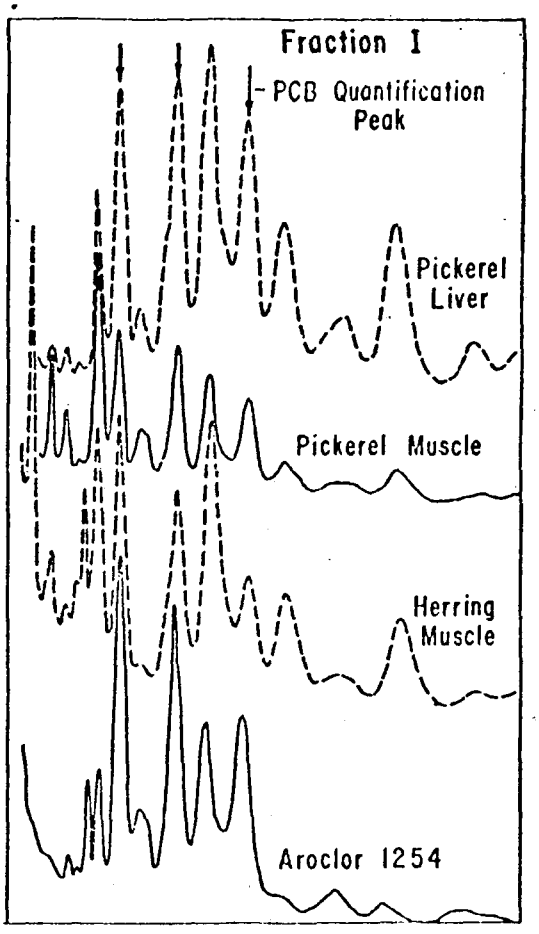

Figure 1. Gas chromatograms of fraction I.

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Untreated pesticide-grade hexane from Fisher Scientific Co. was used and benzene (5 ml/l) was added to Matheson Coleman & Bell pesticide-grade hexane, redistilled in glass, to obtain identical elution patterns (7).

To check the method, commercial corn oil was spiked with Aroclor® 1254 and organochlorine pesticides to contain PCB, hexachlorobenzene (C_6Cl_6), lindane, heptachlor, aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, p,p'-DDD, and p,p'-DDT in concentrations of 0.95, 0.29, 1.95, 1.95, 2.02, 3.81, 5.09, 2.72, 5.70, and 5.70 µg/g, respectively. The average percentage recovery (4 analyses) was 102 (99-105), 78 (77-78), 112 (107-117), 105 (96-115), 113 (112-113), 94 (93-95), 110 (107-113), 113 (110-117), 72 (70-74), and 109 (98-120), respectively.

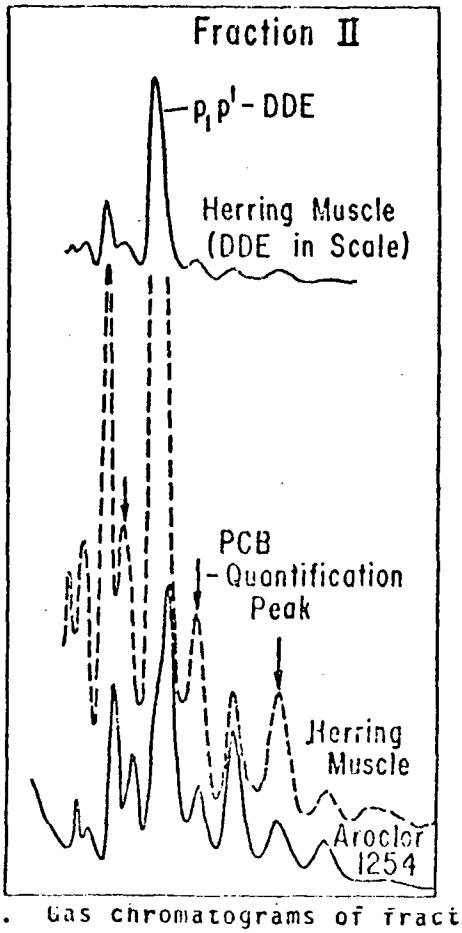

Figure 2. Gas chromatograms of fraction II.

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Three peaks, indicated in Figures 1 and 2 were used to calculate the concentration of PCB in terms of Aroclor® 1254, and the average concentration thus obtained is reported in Table 1. In some samples, containing a relatively high concentration of p,p'-DDE, the middle peak was not clearly separated from that of p,p'-DDE and only the 2 other peaks were used to quantify PCB.

RESULTS AND DISCUSSION

The method simultaneously cleans up the extract and fractionates PCB and organochlorine pesticides. Fraction I contains 70% of PCB, hexachlorobenzene, and some aldrin. Fraction II contains the rest of PCB, heptachlor, and p,p'-DDE, while lindane, heptachlor epoxide, dieldrin, p,p'-DDD, and p,p'-DDT are eluted in fraction III. The major difference between PCB in fractions I and II is the absence of peaks with relative retention times 0.97 and 1.04 (p,p'-DDE = 1.00) from fraction 1. These peaks overlap with the peak of p,p'-DDE in fraction II. In most cases this interference is not significant and a correction of the p,p'-DDE quantification is not necessary.

It is impossible at the moment to quantify accurately PCB in terms of Aroclor® 1254 since the proportions of PCB peaks in the samples are not identical with those in Aroclor® 1254. For example, the total (fractions I and II) ratio of the areas under the first and third PCB quantification peak (Figs. 1 and 2) is 1.01 in Aroclor® 1254, while values of 1.35, 1.88, and 2.81 were found in eel, herring, and mussel, respectively. Error due to differences between commercial PCB and PCB found in the samples is difficult to estimate. It has been suggested that in some cases it may be as high as 100% (1). The precision of the results presented in Table 1, expressed as the difference between duplicate determinations, is 12.3% for eel, pickerel, salmon, herring and mackerel, and 14.6% for mussel. Standard deviation, calculated from all the analyses of eel, is 0.11. For p,p'-DDE the differences between duplicate determinations are 2, 4, 5, 15, 12, and 8% for eel, pickerel, salmon, herring, mackerel, and mussel, respectively. Standard deviations for all the analyses of eel are 0.03, 0.04, 0.16, and 0.004 for p,p'-DDE, p,p'-DDD, p,p'-DDT, and hexachlorobenzene, respectively.

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TABLE I

PCB and organochlorine pesticides in freshwater and marine fishes.

Species	No. of specimens	No. of groups	Av. wt., g	µg/g, wet weight PCB	p,p'-DDE	p,p'-DDD	p,p'-DDT	C_6Cl_6
American eel *(Anguilla rostrata)*	13	4	308	0.71 0.36-1.01 0.57*	0.50 0.44-0.56 0.19*	0.19 0.00-0.37 -	0.29 0.18-0.56 -	0.012 0.006-0.019 0.010*
Chain pickerel *(Esox niger)*	2	1	492	0.33 4.93*	0.16 2.38*	0.03 0.14*	0.06 0.63*	0.003 0.016*
Atlantic salmon *(Salmo salar)*	4	1	1003	0.45	0.22	0.07	0.08	0.002
Herring *(Clupea harengus)*	10	2	222	0.54 0.38-0.73	0.24 0.13-0.30	0.04 0.00-0.08	0.15 0.09-0.23	0.006 0.005-0.006
	10	2	59	0.32 0.20-0.48	0.09 0.07-0.10	0.02 0.02-0.03	0.08 0.06-0.10	0.004 0.003-0.004
	10	2	16	0.34 0.19-0.54	0.06 0.01-0.08	0.01 0.00-0.04	0.05 0.01-0.08	0.003 0.002-0.004
Mackerel *(Scomber scombrus)*	4	1	319	0.35	0.07	0.02	0.07	0.001
Mussel *(Mytilus edulis)*	30	3	30	0.14 0.06-0.25	0.02 0.02-0.03	-	-	-
Cod *(Gadus morhua)*	3	1	1368	0.02	0.01	-	-	-
White fish *(Urophycis tenuis)*	1	1	1035	0.02	0.02	-	-	-
Plaice *(Hippoglossoides platessoides)*	2	1	686	0.03	0.01	-	-	-
Ocean perch *(Sebastes marinus)*	2	1	1267	traces	traces	-	-	-

*liver

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Some of the eels were taken from lakes receiving no industrial effluents or domestic sewage, both identified recently as a source of PCB (8). The levels of PCB found indicate a possibility of pollution by aerial fallout. The occurrence of higher PCB levels in the pelagic species (herring and mackerel) than in groundfish is consistent with this hypothesis. Higher levels of PCB and organochlorine pesticides in herring, average weight 222 g, than in herring, average weight 59 g, obtained from the same location, may indicate a weight-residue relationship similar to that observed for mercury residues in fish (9).

Several unidentified peaks, not corresponding to any found in Aroclor® 1242, 1254, and 1260, were observed in fractions I and II of some samples. A peak, relative retention time 2.85 (p,p'-DDE = 1.00, Fig. 1, pickerel liver, herring muscle), was found in many samples. A peak with the same retention time occurs in Aroclor® 1260.

Of the organochlorine pesticides, p,p'-DDE was present in highest concentrations, followed by p,p'-DDT and p,p'-DDD. A small peak with the retention time of dieldrin was found in pickerel liver. Most of the samples contained a small amount of hexachlorobenzene. It is likely that some of the recently reported levels of DDT and metabolites in Canadian Atlantic fishes (3) are too high due to interference of PCB.

In terms of acute toxicity, PCB are less toxic to fish than organochlorine pesticides (10). Little is known about the sublethal effects of PCB and it is not possible at the moment to make any conclusions about the toxicological significance of the levels of PCB found in fish. In view of the recently reported high levels of PCB in human milk and adipose tissue (11), and the identification of toxic chlorinated dibenzofurans in some commercial PCB preparations (12), more knowledge of the distribution and effects of PCB in the environment is needed.

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