20 November 1975

Session IV:

ECOLOGICAL EFFECTS AND EXPOSURE

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INTRODUCTORY REMARKS

Donald I. Mount, Ph.D.

Not being a back-of-the envelope modeler like Dr. Nisbet, I am not going to try to play the role that he did. But I do think there are two or three very quick comments that might help in moving into our discussion.

First of all, it is worth pointing out that there are three primary reasons to be concerned about the impact of pollutants on aquatic organisms. First and most obvious are the effects of pollutants on the organisms themselves. Perhaps less obvious is the warning that when residues accumulate in aquatic organisms, potential hazards to human health may exist.

We have heard about the impact of residues in human food on humans. I think it is worth emphasizing that essentially 100 percent of the organism's diet has residues unlike those in humans. Therefore, the significance of a given concentration of PCB's in the food of an animal such as the mink may be quite different than it is to a human.

I had talked with Dr. Nisbet before the conference and we agreed in the upcoming session to discuss residues only where they relate to effects on the organisms carrying them.

I have noted as I have sat in the audience that it has been very difficult to tell whether the speaker is saying parts per billion or parts per million. So I have asked our speakers if they can refer to micrograms per liter and milligrams per liter. For those who cannot make the conversion, that is parts per billion and parts per million, respectively. So, if a speaker says milligrams per liter, that is parts per million.

I would like now to start with our papers and we would like to reverse the position of the first two papers, because the first one is to deal with the information that was available up to 1972 regarding the effects of PCB's in nonhuman organisms. We are referring, of course, to those organisms in the environment more so than domestic animals. If you wonder why 1972 was picked, it correlates roughly with the report of the PCB task force.

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SUMMARY OF RECENT INFORMATION REGARDING EFFECTS OF PCB's ON BIRDS AND MAMMALS

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Abstract

The significance of PCB's to wild animals depends both upon their lethal toxicity and their sublethal physiological effects. The lethal dietary toxicities of most Aroclors to experimental birds generally are less than those of DDE, DDT, or dieldrin. Effects of PCB's on reproduction are apt to have the most serious impact on populations. These effects are difficult to evaluate. The chief reproductive effects in chickens are reduced egg production and hatchability. Deformities in chicks are common, and growth rates of young sometimes are depressed. Administration of low dietary levels of PCB's to chickens, bobwhite quail, ringdoves, and mallards have not resulted in thinning of eggshells. No tests of shell thinning have been made with birds of prey or other critical species. Residues of PCB's in wild birds often are at levels known to have caused reproductive impairment in chickens. Species differences must be considered, however, and the significance of these levels to wild birds is very uncertain.

PCB's have been implicated in the reproductive failure and mortality of mink from the Great Lakes region. High levels of PCB's and organochlorine pesticides have been found in marine mammals and have been linked with premature births in some species. Recent studies have shown that some species of bats may be sensitive to a low level of PCB contamination.

INTRODUCTION

The widespread distribution of PCB's in the environment and their effects upon populations of wild birds and mammals are matters of growing concern. Their presence has stimulated research to evaluate their role in the biosphere. This paper reviews recent literature (1972-1975) on the effects of PCB's upon birds and mammals.

EFFECTS ON BIRDS

Lethal Toxicity

Outright mortality can affect populations. Measurement of direct toxicity is an important first step in the evaluation of a chemical. Toxicities of different PCB's to young pheasants (*Phasianus colchicus*), mallards (*Anas platyrhynchos*), bobwhite quail (*Colinus virginianus*), and coturnix quail (*Coturnix coturnix*) have been compared with the toxicities of DDT, dieldrin, and other insecticides (ref. 1). LC_{50}'s for the PCB's were high. Tests of six PCB mixtures, containing 32 to 62 percent chlorine, showed that the toxicity increased with the percentage of chlorine. The dietary toxicities of the Aroclors were, with few exceptions, less than those of the four organochlorine pesticides used for comparison. Special tests with coturnix quail showed that the toxic effects of DDE and Aroclor 1254 were additive but not synergistic. In other studies, Aroclor 1254 was approximately as toxic as DDE to four species of blackbirds (ref. 2). Redwinged blackbirds (*Agelaius phoeniceus*) were somewhat more susceptible to DDE than to PCB's.

Regular oral doses from 10 to 210 mg Aroclor 1254 produced some mortality among subadult pheasants (ref. 3). The amount and timing of the mortality were related to the dose. Heavier birds lived longer and lost the greatest percentage of their body weight before death.

Residues of PCB's in brains of blackbirds given heavy doses of Aroclor 1254 were diagnostic of death (ref. 4); residues in brains of birds that died varied from 349 to 763 ppm (wet weight) and were not above 301 ppm in survivors. In studies with pheasants given daily doses of 210 mg of Aroclor 1254, a brain residue level of 300 to 400 ppm generally indicated death due to PCB toxicosis (ref. 3). Residues of PCB's in livers and other tissues were more variable and of lesser value for diagnosing death.

PCB residues in brains of bald eagles (*Haliaeetus leucocephalus*) found dead from various causes in the United States between 1969 and 1972 varied from 0.10 to 230 ppm (refs. 5,6). Even the highest levels in the eagle brains were below the lethal range determined with captive blackbirds. However, there may be at least two distinct modes of death from PCB's (ref. 7). A sudden heavy intake may cause death from neurotoxicity, with brain residues high and diagnostic of death. Long, low intake, which may occur in the field, may kill by causing edema and related phenomena; signs vary between individuals and between species. Under these circumstances, mortality resulting from PCB's would not necessarily be accompanied by high residues of PCB's in brains, and for these birds no good diagnostic technique is available, for the signs are nonspecific and the residue levels vary greatly.

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PCB's were implicated in a dieoff of guillemots (*Uria aalge*) which occurred in the Irish Sea region in 1969 (ref. 8). The body load of PCB's in birds that died was about twice that in healthy birds collected in the same general region. Moreover, livers of shot birds contained only 0.9 percent of the total body burden compared to 22.5 percent for birds found dead. PCB residues in brains were not determined.

Uptake and Loss Rates

PCB's are readily taken up by animal tissues, and loss rates are reasonably slow. Like DDT and other lipid-soluble pesticides, PCB's accumulate in adipose tissue and have been shown to move out of adipose tissue during lipid mobilization. In experimentally dosed birds, highest PCB levels accumulated in adipose tissue, followed by kidney, liver, brain, muscle, and blood (refs. 3,9).

In experimentally dosed grackles (*Quiscalus quiscula*), half of the PCB's were gone from the tissues in about 1 month, compared to an estimated 6 months for DDE (ref. 4). Continued loss of PCB's after the first month, however, was exceedingly slow and there was no significant further loss occurring during the next 12 weeks. After 32 weeks, about 16 percent of the original burden of PCB's remained in the body.

PCB's are readily excreted in the egg. PCB levels in the eggs of double-crested cormorants (*Phalacrocorax auritus*) reflected carcass levels, but this relationship did not hold for white pelicans (*Pelecanus erythrorhynchos*) (ref. 10). In ringdoves (*Streptopelia risoria*), Aroclor 1254 fed at 10 ppm reached an equilibrium level of 17 ppm wet weight in the eggs; this was about 2 percent of the level found in the fat of the adult birds (ref. 9).

PCB's stored in the adipose tissue are readily mobilized during periods of stress that cause loss of body weight. Alternate starving and feeding of pheasants dosed with PCB's increased the toxicity of the PCB's (ref. 11). Birds that died from starvation had mobilized their fat reserves and had considerably higher PCB's in the brain and other tissues than did PCB-treated birds that were not starved. Similarly, increased levels of PCB in muscle, brain, and liver of PCB-treated ringdoves followed depletion of fat reserves by starvation. At death, PCB concentration in the brains of starved birds had increased 56-fold (ref. 9).

Reproductive Effects

Effects of PCB's on reproduction are difficult to evaluate but are apt to have the most serious impact on populations. Few data are available for wild species, so work done with chickens must be relied upon. Numerous good studies have shown that chickens are sensitive to PCB's. The chief reproductive effects in chickens are lowered egg production and reduced hatchability. Deformities in chicks are common, and growth of young may be depressed. Survival of young sometimes is affected. Feed consumption, adult survival and body weight, eggshell characteristics, and gametogenesis usually are not affected. Most embryo mortality occurs during the latter stages of incubation, although this may depend upon the PCB residue in the egg. Many chicks may die after pipping. The most severe effects are produced by Aroclors in the middle of the range—from 1232 through 1254.

Chickens fed 2 ppm of several Aroclors in the diet for 9 weeks were not adversely affected (ref. 12). Others fed 20 ppm were affected differently by the different types of Aroclors. Aroclors 1221 and 1268 did not affect egg production or hatchability. Aroclor 1248 produced the most severe effects, causing some mortality of adults and nearly eliminating hatching of eggs. Aroclor 1242 produced effects that were very nearly as severe. Aroclor 1232 produced less effect.

The lack of effects of 2 ppm of dietary PCB on chicken reproduction was supported by a 39-week feeding test with Aroclor 1254 (ref. 13). Five ppm of 1254 reduced egg production erratically over the 39-week period. With 50 ppm, however, mortality began, and dosage was stopped after 14 weeks. Egg production fell sharply. Hatchability dropped nearly to zero; residues in eggs were then about 25 to 50 ppm. As residues in eggs dropped after 6 weeks of clean food, hatchability rose. Residues over 10 to 15 ppm in eggs were accompanied by heavy embryotoxicity, but those below 5 ppm showed no effect. At the start of the 50-ppm dosage, deaths of embryos came late in development, but as residues built up deaths came progressively earlier.

The effects on reproduction were further investigated in another study in which chickens were given 50 ppm Aroclor 1254 in water for 6 weeks, and then were given untreated water for 20 weeks (ref. 14). Hatchability dropped to zero within 3 weeks after dosage began and stayed nearly at zero through 8 weeks of clean water, then rose to approximately normal levels after 16 weeks of untreated water. The amount of embryonic mortality associated with a given amount of PCB residue in the egg became higher as the study progressed. Thus, 50 percent mortality of embryos was correlated with 50 ppm of PCB in the yolk after 1.6 weeks of dosage, but with 10 ppm after 18.7 weeks, a fivefold difference. The

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greatest toxicity per unit of PCB came after 11 weeks of untreated water. Late in the study, 6 to 8 ppm of PCB in yolks was correlated with 14 to 36 percent mortality of embryos. This would represent about 3.6 ppm in the whole egg. The authors concluded that the increase of toxicity with time was caused by the accumulation of some persistent isomer or homolog of 1254 or by a metabolite.

In another study with chickens, egg production was reduced by 10 percent and hatchability by 44 percent when eggs from hens dosed with Aroclor 1248 contained only 3 ppm (ref. 15). When levels in the eggs reached 4.5 ppm, production was further reduced and hatchability was almost completely eliminated.

PCB residues in eggs of wild birds have often equalled or exceeded the levels known to have caused reproductive problems in chickens. For a few examples, 11 eggs of bald eagles from the United States contained from 2.2 to 27.7 ppm of PCB's with a median of 9.7 ppm (ref. 18). PCB residues in osprey (*Pandion haliaetus*) eggs collected in Connecticut in 1968 and 1969 varied from 3.6 to 51 ppm (ref. 17) and brown pelican (*Pelecanus occidentalis*) eggs from the Eastern United States contained from 1.9 to 36.5 ppm PCB's, with many readings over 5 ppm (ref. 18). Species differences in response must be considered in the interpretation of the effects of these levels upon reproduction of wild birds. The number of species that may respond like chickens is not known.

PCB's caused deformities in chicks from hens dosed with 50 ppm Aroclor 1254 (ref. 19). Many deformities appeared when residues in yolks were 10 ppm or more. In another study in which hens were dosed with 20 ppm of Aroclors 1232, 1242, 1248, or 1254, 34 percent of 843 embryos that died during incubation showed signs of abnormal development (ref. 20). PCB's or related chemicals were suspected of causing deformities in a small number of young in a tern colony in which PCB residues were high (ref. 21).

Other species of birds may be less sensitive to PCB's than chickens. Mallards showed no decline in reproductive success during 2 years of dosage with 25 ppm of Aroclor 1254 (ref. 1). Similar results have been reported for coturnix quail (refs. 15,22) and bobwhite quail (ref. 1). Pheasants given oral doses of Aroclor 1254 showed depressed egg production and hatchability, suggesting a response similar to that of chickens (ref. 23).

Decreases in eggshell thickness and associated declines in populations of certain species of wild birds have been linked with elevated levels of DDE in their eggs (for review see ref. 24). The effect of DDE upon eggshell thickness has been demonstrated experimentally with several species (ref. 24). Recent experimental studies with PCB's, however, have failed to demonstrate that low dietary dosages cause significant eggshell thinning in mallards (ref. 1), bobwhite quail (ref. 1), pheasants (ref. 23), ringdoves (ref. 25), or chickens (refs. 12,15,26,27). In earlier tests, chickens fed Aroclor 1242 at 10 ppm or 100 ppm and Aroclor 1254 at 100 ppm laid eggs with thinner shells than did controls (ref. 28). Coturnix quail hens given 10 ppm Aroclor 1242 in the feed for 40 days produced eggs with shells 5 percent thinner than did hens given untreated feed (ref. 29). Similarly, 50 ppm of Aroclor 1254 in the diet produced a small decrease in shell thickness and an increase in the percentage of cracked eggs of coturnix quail (ref. 22). No tests of eggshell thinning have been made with birds of prey or other critical species.

Statistical evaluation of the role that different chemicals may play in thinning the eggshells of brown pelicans in the field has shown that DDE residues correlate much better with shell thinning than do residues of PCB's (ref. 18). Similar relationships have been shown for other species (ref. 30).

Effect on Chromosomes

Few cytogenetic studies have been conducted to determine effects of PCB's on chromosome structure. In one study, no chromosomal aberrations were detected in chicken embryos following injection of Aroclor 1242 into the egg to levels of 10 and 20 ppm (ref. 31). At these levels mortality of embryos was high. In another study, embryos from ringdoves treated with Aroclor 1254 at 10 ppm had a higher frequency of chromosome aberrations than did controls (ref. 32).

Chromosomal aberrations may contribute to the abnormalities of developing embryos observed in some studies, or perhaps to a decreased reproductive success during successive generations of exposure to PCB's. When ringdoves were exposed to 10 ppm Aroclor 1254 during two generations, the reproductive success of the first generation was normal, but the success of the second generation was greatly reduced (ref. 32).

Effects on Behavior

Alterations in behavior may bring about depressed survival and reproductive success of wild species. Experimental studies have shown that PCB's may alter behavior. Caged European robins (*Erithacus rubecula*) fed PCB's showed increased migratory activity compared with untreated controls (ref. 33). PCB's caused a similar tendency in redstarts (*Phoenicurus phoenicurus*) (ref.

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34). Behavior, on a visual cliff, of pheasant chicks hatched from hens given 50 mg Aroclor 1254 weekly was significantly different from controls or those receiving 12.5 mg (ref. 23); more chicks from the 50-mg group jumped to the visually deep side or made no choice of sides than chicks from other groups. The avoidance response to a moving silhouette of coturnix quail chicks fed 200 ppm of Aroclor 1254 was greatly reduced (ref. 35); there was no significant recovery in response after the birds were again given clean feed, suggesting an effect upon the maturing central nervous system.

Embryonic mortality of ringdoves fed 10 ppm Aroclor 1254 was higher when the eggs were incubated by the parent birds than when they were incubated artificially (ref. 36). Egg temperatures suggested that the increased mortality was due to decreased parental attentiveness.

EFFECTS ON MAMMALS

Certain species of mammals appear to be especially sensitive to ingestion of low levels of PCB's. An extreme example was provided by the declining reproductive success and increasing mortality of mink that was observed by commercial mink ranchers in the Great Lakes region in the 1960's. The problems developed when mink ranchers began to use coho salmon from the Great Lakes in mink rations (ref. 37). Other fish from Lake Michigan had similar effects, but coho from the Pacific coast caused no trouble. Feeding trials were conducted with several contaminants that had been identified in the fish (ref. 38). Neither DDT nor dieldrin caused these effects in mink, even at levels far higher than those in the fish. In the first test, a mixture of PCB's at 30 ppm in the diet killed all the adult mink. With dietary dosages of 5 and 10 ppm of Aroclor 1254, no reproduction occurred. At 10 ppm, five of the six adults died. In further tests, 1 ppm of PCB in the diet reduced reproductive success. More drastic results were observed when the mink were fed meat from cows that had been dosed with Aroclor 1254 (ref. 39). When the concentration of PCB in the diet was 3.57 ppm, there was no reproduction and all breeders died. When the concentration was 0.64 ppm, some adults died and no surviving young were produced.

High levels of PCB's and organochlorine insecticides have been found in marine mammals and a link with premature births in some species has been suggested. Premature pups from California sea lions (*Zalophus californicus*) contained higher levels of these compounds in the blubber, liver, and brain than did full-term pups (ref. 40).

Recent studies have shown that certain species of bats are sensitive to PCB's. In big-brown bats (*Eptesicus fuscus*), PCB's crossed the placenta 2 to 3 times more readily than DDE (ref. 41). Concentrations of PCB's were significantly greater in litters that included dead young than in litters in which both young were born alive. In further studies, big-brown bats fed 250 ppm DDE in the diet gained weight and none died, but individuals fed only 10 ppm Aroclor 1264 gained weight more slowly and some died (ref. 42).

PCB's have been shown to be toxic to some nonhuman primates over a wide dose range. Adult rhesus monkeys developed signs of PCB intoxication within 1 to 2 months at doses as low as 2.5 and 5.0 ppm of Aroclor 1248 in the diet (ref. 43); these levels also caused a marked decline in reproductive success.

CONCLUSIONS

The effects of PCB's upon wild populations are difficult to evaluate and many important questions remain to be answered. Residues in wild species may be derived from different commercial mixtures that have undergone metabolic changes for varying time periods before reaching the target organism. Residues of PCB's in wild birds often are at levels known to have caused reproductive impairment in chickens. Nevertheless, species differences must be considered and it is not known how many species respond in the same manner as chickens. The great sensitivity of mink raises the question of how many other mammals may be equally sensitive to reproductive impairment or mortality.

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PRE-1972 KNOWLEDGE OF NONHUMAN EFFECTS OF POLYCHLORINATED BIPHENYLS

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Abstract

Research conducted worldwide on the effects of PCB on aquatic organisms, birds, and mammals is herein summarized. The ecological effects of PCB are still not fully understood, nor is the long-term impact of PCB on animal populations. It is known that PCB are highly persistent contaminants that bioaccumulate in the food chain. They produce both chronic and acute effects on the growth, reproduction, and behavior of fish, birds, and mammals. Of these, freshwater fish accumulate PCB to the greatest extent. The birds and mammals that feed on freshwater fish in turn take in hazardous amounts of PCB residues and become subject to their serious toxicological effects.

INTRODUCTION

Polychlorinated biphenyls were under investigative development as early as 1881 (Schmidt and Schultz) and yet very little knowledge of their toxic effects was evident until they had gained wide use during the 1930's and pathological studies on laboratory animals were conducted (refs. 5,41). These polychlorinated biphenyls, napthlenes, terpenes, etc., were marketed under a variety of trade names–Aroclor, Chlorphen, Kanechlor, Phenochlor–and a series of numerical synonyms such as we have become familiar with in Aroclors–1221, 1232, 1242, 1248, 1254, 1260, 1268, etc. (refs. 8, 33, 51).

Almost 20 years lapsed until McLaughlin et al. reported that Aroclor 1242 was highly toxic and produced teratogenic effects in chick embryo at low dosage levels (ref. 44). Many studies were initiated at about this time, when analytical chemists monitoring environmental components observed a number of unidentified peaks in gas chromatographic analysis that were most frequently associated with aquatic species of birds and fishes (refs. 3, 19, 20, 21, 22, 24, 25, 26, 27, 32, 33, 34, 45, 46, 48, 53, 54, 55, 56, 58, 59, 61, 64, 66, 67, 68, 83, 84, 85, 86, 88, 93, 94). The annual research reports by the U.S. Fish and Wildlife Service since 1969 gave technical details in the developments of our work on the toxicity of PCB and chlorinated hydrocarbon pesticides, which coincided with monitoring investigations of persistent chemicals such as DDT (refs. 3, 12, 16, 17, 38, 39, 40, 47, 52, 57, 71, 77, 78, 79, 80, 81, 82, 89, 90, 91, 92). The development of the silicic acid method allowed for the separation of PCB from other pesticide components and permitted detailed investigation of these environmental contaminants (refs. 1,71).

The December 1971 conference on polychlorinated biphenyls sponsored by the National Institute of Environmental Sciences at the Quail Roost Conference in Rougemont, North Carolina, provided full array of the effect of PCB on experimental animals, fish, and wildlife. The Fish and Wildlife Service National Pesticide Monitoring Program discussed at this conference concentrations of PCB in fish samples (ref. 89). These residue levels were confirmed in the cross-check analysis by the Fish Pesticide Research Laboratory, which in turn has spurred more toxicological research into the significance of these residues (refs. 18, 40, 71, 74, 75, 76, 80).

EFFECTS ON AQUATIC ORGANISMS

Chronic and acute toxicity studies on aquatic organisms were conducted in which 96-hour LC_{50} values were determined for eight of the Aroclor series from 1221 through 1268 (refs. 40,71). The 96-hour LC values ranged from 1,170 to 50,000 mg/l for cutthroat trout (table 1) (refs. 40,71). In another test, the acute oral toxicities of Aroclors 1242, 1248, 1254, and 1260 were found to be greater than 1500 mg/kg in rainbow trout. Intermittent flow for Aroclors 1242, 1248, and 1254 indicated that these compounds were much more toxic at high temperatures and at longer exposure periods. Comparable results were obtained on channel catfish and rainbow trout (table 1) (refs. 40,71).

The effect of Aroclors on invertebrate forms gave an even wider distribution of effects (table 2) (refs. 40,69,71), with Aroclor 1248 being the most toxic to *Daphnia magna* and levels above 5 mg/l causing inhibition in reproduction (ref. 45). Similar results were obtained on *Gammarus pseudolimnaeus* (ref. 45). The 96-hour LC_{50} values for Aroclors 1248 and 1254 were 52 and 2400 mg/l respectively (ref. 40). Another species of scud (*Gammarus fasciatus*) was found to concentrate PCB by 27,000 times the exposure level compared to the *Daphnia*, which concentrated Aroclor 1254 by 48,000 times (figure 1) (ref. 40).

Both crayfish and the scud were found to be much more sensitive to Aroclor 1242, with the uptake being

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NOTE: Scientific names for fish and wildlife listed in text are given at the end of the paper.

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Table 1. Toxicity of PCB to fish (refs. 40,71)

PCB	Invertebrate (species)	Bioassay method[1/]	Exposure time	LC_{50} (µg/g)
Aroclor 1242	Scud (*Gammarus faciatus*)	C	4	10.0
	Scud (*Gammarus faciatus*)	C	10	5.0
	Crayfish (*Orconectes nais*)	A	7	30.0
	Damselfly (*Ischnura verticalis*)	A	4	400.0
	Dragonfly (*Macromia sp.*)	A	7	800.0
Aroclor 1248	Scud (*Gammarus faciatus*)	A	4	52.0
Aroclor 1254	Scud (*Gammarus faciatus*)	A	4	2400.0
	Glass shrimp (*Palaemonetes kadiakensis*)	C	7	3.0
	Crayfish (*Orconectes nais*)	C	7	80.0
	Damselfly (*Ischnura verticalis*)	C	4	200.0
	Dragonfly (*Macromia sp.*)	A	7	100.0
2,4'-Dichloro-biphenyl	Scud (*Gammarus pseudolimnaeus*)	A	4	120.0
4,4'-Dichloro-biphenyl	Scud (*Gammarus pseudolimnaeus*)	A	4	100.0
2,3,4'-Trichloro-biphenyl	Scud (*Gammarus pseudolimnaeus*)	A	4	70.0
2,4,5,2',5'-Penta-chlorobiphenyl	Scud (*Gammarus pseudolimnaeus*)	A	4	210.0
2,4,6,2',4',6'-Hexa-chlorobiphenyl	Scud (*Gammarus pseudolimnaeus*)	A	4	150.0

1/ A = Acute toxicity by static test procedure
C = Chronic toxicity by flow-through procedure

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Table 2. Toxicity of PCB to aquatic invertebrates (refs. 40,69,71)

PCB (Aroclor)	Fish	Temp (°C)	Bioassay method[1/]	LC_{50} value in µg/l at exposure time of						
				4d	5d	10d	15d	20d	25d	30d
1221	Cutthroat trout	8.9	A	1170	--	--	--	--	--	--
1232	Cutthroat trout	8.9	A	2500	--	--	--	--	--	--
1242	Cutthroat trout	8.9	A	4530	--	--	--	--	--	--
	Rainbow trout	17.0	C	--	67	48	18	10	12	--
	Bluegill	20.0	C	--	154	72	54	--	--	--
	Bluegill	17.0	C	--	--	--	164	125	120	84
	Channel catfish	20.0	C	--	--	174	107	--	--	--
	Channel catfish	17.0	C	--	--	--	219	150	132	87
1248	Cutthroat trout	8.9	A	5750	--	--	--	--	--	--
	Rainbow trout	17.0	C	--	54	38	16	6.4	3.4	--
	Bluegill	18.3	A	278	--	--	--	--	--	--
	Bluegill	20.0	C	--	307	160	76	10	--	--
	Bluegill	17	C	--	136	115	111	106	100	78
	Channel catfish	18.3	A	6000	--	--	--	--	--	--
	Channel catfish	27.0	C	--	--	94	57	--	--	--
	Channel catfish	20.0	C	--	--	225	127	--	--	--
	Channel catfish	17.0	C	--	--	121	121	115	104	75
1254	Cutthroat trout	8.9	A	42500	--	--	--	--	--	--
	Rainbow trout	20.0	C	--	156	8	--	--	--	--
	Rainbow trout	17.0	C	--	--	160	64	39	27	--
	Bluegill	18.3	A	2740	--	--	--	--	--	--
	Bluegill	20.0	C	--	--	443	204	135	54	--
	Bluegill	17.0	C	--	--	--	303	260	239	177
	Channel catfish	18.3	A	12000	--	--	--	--	--	--
	Channel catfish	20.0	C	--	--	--	741	300	113	--
	Channel catfish	17.0	C	--	--	303	286	293	181	139
1260	Cutthroat trout	8.9	A	60900	--	--	--	--	--	--
	Rainbow trout	20.0	C	--	156	5	--	--	--	--
	Rainbow trout	17.0	C	--	--	326	143	78	49	51
	Bluegill	20.0	C	--	--	--	--	245	212	151
	Bluegill	17.0	C	--	--	--	--	--	--	400
	Channel catfish	20.0	C	--	--	--	--	296	166	137
	Channel catfish	17.0	C	--	--	535	482	512	465	433
1262	Cutthroat trout	8.9	A	50000	--	--	--	--	--	--
1268	Cutthroat trout	8.9	A	50000	--	--	--	--	--	--

1/ A= Acute toxicity by static test procedure.
C= Chronic toxicity by flow-through procedure.

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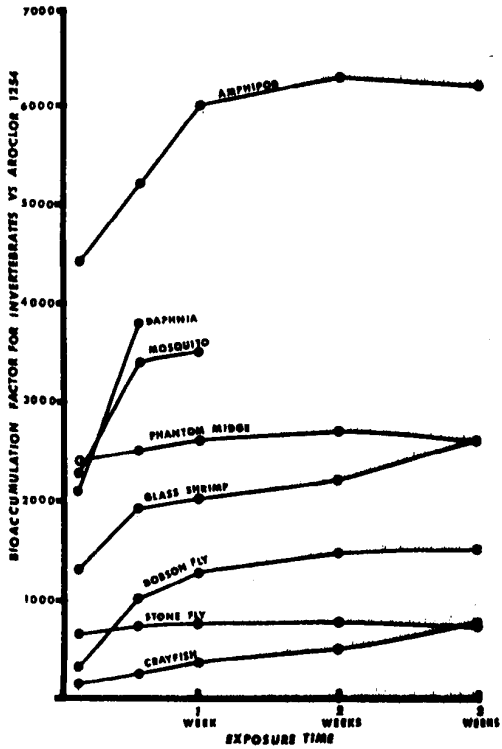


Figure 1. Bioaccumulation of Aroclor 1254 for various aquatic invertebrates exposed to water concentrations: 1.1 µg/l for *Daphnia magna*; 1.8 µg/l for *Gammarus pseudolimnaeus*; 1.2 µg/l for *Orconectes nais*; 1.3 µg/l for *Palaemontes kadiakensis*; 2.8 µg/l for *Pteronarcys dorsata*; 1.1 µg/l for *Corydalus cornutus*; 1.5 µg/l for *Culex tarsalis*; 1.3 µg/l for *Chaoborus punctipennis* (ref. 40).

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somewhat enhanced with time. Glass shrimp *(Palaemonetes kadiakensis)* were also found to be quite sensitive to Aroclor 1254, with an LC_{50} value of 3 mg/l in a 7-day exposure. On the other hand, dragon fly (*Macromia* species) and damsel fly (*Ischnura verticalis*) were a great deal more resistant to Aroclors 1254 and 1242 (table 1 and figure 1).

Aroclor 1254 was found to be extremely toxic to immature pink shrimp, of which more than 50 percent died within 15 days of continuous exposure to 0.94 mg/l (ref. 47). A similar concentration factor was noted for Aroclor 1254 in two estuarine fish species (*Lagondon rhomboides* and *Leiostomus xanthurus*). While between 14 and 45 days of exposure to 5 mg/l did product some mortality, the 1 mg/l during the same exposure time resulted in no mortality, but concentration of residues did occur at rates similar to those for freshwater fish—10,000 to 50,000 times the environmental levels (refs. 12,16).

In studies conducted in Sweden on salmon eggs, PCB residues on a lipid basis ranging from 7.7 to 34 µg/g caused mortalities between 16 to 100 percent (ref. 26). The regression analysis gave a coefficient of correlation of 0.85, with a significance of P = .001. PCB residues in these Atlantic salmon eggs on a wet weight basis were from 0.4 to 1.9 µg/g. This indicates that the threshold for egg mortality was about 0.5 µg/g PCB. Such residues would be comparable to whole fish residues of 2.5 to 5.0 µg/g or very close to those values found in many fish sampled in our National Pesticide Monitoring Program and in Sweden (refs. 6,89).

The oral toxicities of Aroclors 1242, 1248, 1254, and 1260 were found to be in excess of 1500 µg/kg for adult rainbow trout (ref. 40). However, trout fry from eggs containing 2.7 µg/g of Aroclor 1242 along with a DDT residue of 0.09 µg/g caused a 75 percent cumulative mortality 30 days after hatching (ref. 18). In five other groups of less contaminated eggs, 10 to 28 percent mortality was noted 30 days after hatching. About 60 to 70 percent of the fry that survived were deformed and teratology was evident. Generally speaking, the more chlorinated PCB were not as acutely toxic as the less chlorinated ones. For example, in cutthroat trout, Aroclors 1221, 1232, 1242, 1248, 1254, and 1260 had LC_{50} values for 96-hour exposure of 1.2, 2.5, 5.4, 5.7, 42, and 61 mg/l respectively.

In tests with individual homologs, toxicity decreases as the percentage of chlorine increases in amphipods against 5 PCB formulations (ref. 40). The dichlorobiphenyls and trichlorobiphenyls dominate the Aroclor 1242, whereas the trichlorobiphenyls and tetrachlorobiphenyls dominate in Aroclor 1248 and the pentachlorobiphenyls and hexachlorobiphenyls dominate in the Aroclor 1254. The most toxic PCB formulations appear to be those that contain 4 to 5 chlorine atoms; these predominated in Aroclor 1248, which was found to be the most toxic of all of the Aroclors tested on fish. A noticable shift in residue components following exposure to Aroclor 1254 was observed in experiments conducted on amphipods (ref. 71). An apparent twofold increase in concentration of less chlorinated isomers and homologs occurred in the trichlorobiphenyls and tetrachlorobiphenyls. The higher chlorinated pentachlorobiphenyls and hexachlorobiphenyls were decreased by half in experiments conducted on fish (ref. 40). Tests showed that residue accumulation of PCB in invertebrates ranged from 160 to 6,300 times that in water. In channel catfish, after 77 days of exposure, the concentration of Aroclors 1248 and 1254 accumulated in channel catfish to 56,270 and 61,190 times, respectively, than in water (figure 1). The more chlorinated components accumulated more than the less chlorinated ones, and upon termination of the exposure, PCB elimination by fish was more rapid for the less chlorinated components (refs. 40,71). The researchers also found evidence that the actual uptake is less or the turnover rate is more rapid in the invertebrates. This is in agreement with observations by other investigators (refs. 48, 64, 65, 66, 67).

In other experiments conducted by our Fish Pesticide Research Laboratory, we found that inclusion of Aroclor 1254 in fish food at the rate of 0.448 µg/g resulted in a 52 percent increase in fish thyroid activity. In a high-treatment group that received 480 µg/g, thyroid stimulation was 119 percent over that of the control group (figure 2) (ref. 40). In these tests we found that the lowest detectable stimulation thyroid activity occurred in salmon dosed at 1/1,000th the dosage that would cause mortality. This test was further duplicated in channel catfish with comparable results, which suggests that the laboratory studies may be realistic indicators of the physiological effects of PCB on fish and the aquatic environment (figure 3) (refs. 40,71). The true effects of thyroid stimulation, however, on respiration, carbohydrate metabolism, oxygen consumption, ammonia metabolism, osmoregulation, growth, oxidative phosphorylation, central nervous system function, and behavior of fish are yet to be determined. Nevertheless, we can postulate that these must be important and interrelated factors in the physiological and biochemical function of fish and any alteration of this normal function could effect fishes' ability to adapt to stress or such changes in the environment as salinity and temperature. The effect of environmental stress on the health of fish is very well illustrated by Snieszko (figure 4) (ref. 70). Subtle effects

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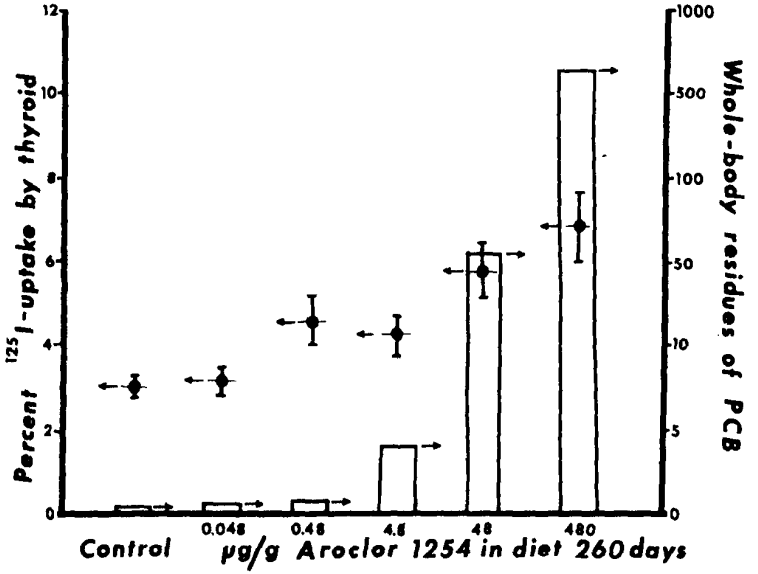


Figure 2. Whole-body residues and thyroidal uptake of ^{125}I in coho salmon fed diets containing Aroclor 1254 for 260 days (*ref.* 40).

on microflora, fauna, and plankton must also be investigated for potential impacts on trophic level ecology (refs. 29,30).

Enzymatic and compositional changes of the microsomes occur where possible lipids are concentrated in the membrane and manifestation of enzyme malfunction is suspect in the proximity of hydrophobic chlorinated aromatic hydrocarbons such as PCB, DDT, and other organochlorine pesticides that would accumulate (*ref.* 50). Mayer, Mehrle, and Sanders conducted experiments with incorporation of Aroclor 1248 into the diet of juvenile lake trout in concentrations of 0.2 to 6 mg/kg of food (ref. 40). These levels, comparable to those found in natural food, suppressed growth and serium cortisol levels, but stimulated thyroid activity during the first 160 days of the experiment (*ref.* 40). After 320 days, however, control and treated fish were similar.

Histopathological examination of fish exposed to Aroclor and chlordane showed a very high incidence of gill lesions after 6 months exposure; 6 percent of the PCB-treated fish were affected, compared to 15 percent after 9 months (*refs.* 76-78). Progressive and diffuse degeneration changes in liver were noted in 80 percent of the fish after 4 to 6 months exposure to the chlordane or PCB diet. A nonspecific degeneration of liver pyrenchema and cytoplastic vaculation of liver cells occurred in addition to pleomorphism. The severity of liver tissue degeneration throughout the 8- to 9-month exposure period exceeded the controls by at least threefold. After this, we noted that focal areas in liver degeneration decreased in size and by the following year no liver lesions could be observed in this lot of fish. This observation was complemented by the decrease in severity of gill lesions in PCB-treated fish. The gill lesions also improved after 9 to 12 months of exposure and no significant difference between treated and control fish could be detected the following year.

In parallel experiments, whole-body residues had

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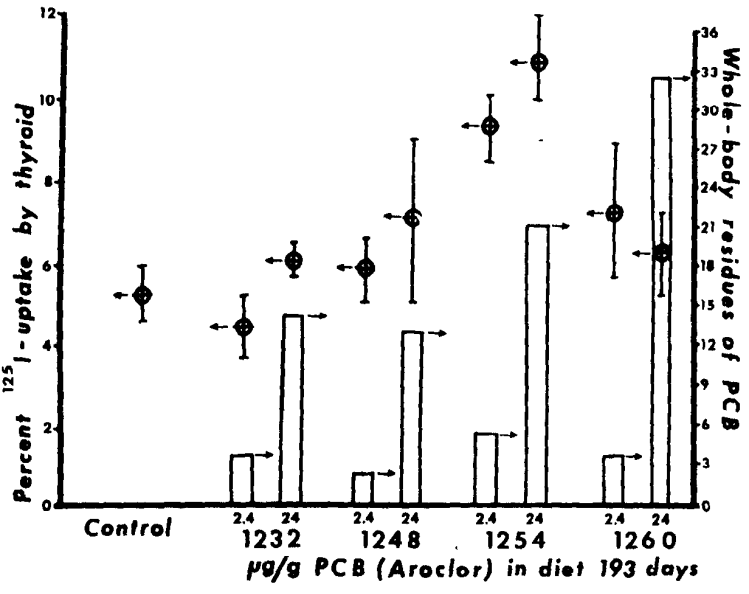


Figure 3. Whole-body residues and thyroidal uptake of ^{125}I in channel catfish fed diets containing Aroclors 1232, 1248, 1253, and 1260 for 193 days (ref. 40).

increased from 0.13 mg/kg at the lower dosage of 0.1 mg/kg in the food to a residue of 9.7 mg/kg at the higher food dosage 6 mg/kg after 320 days (ref. 40). After the trout were fed uncontaminated food for 60 days, the residues declined an average of 31 percent, 15 percent, and 12 percent in the 0.6-, 1.8-, and 6.0-mg/kg treatments respectively. Lake trout growth was retarded by the dietary intake of Aroclor 1248. These results suggest that although fish have a remarkable ability to recuperate and regenerate tissue, the extent and lethality of damage through intoxication by PCB are not clear. However, precise toxicological implication of Aroclors on the general well being and health of fish is certainly suspect (ref. 70).

EFFECTS ON BIRDS AND MAMMALS

Behavioral effects of organochlorine pesticides and PCB have been noted in the studies conducted on Japanese or coturnix quail fed sublethal concentrations and subjected to a simultaneous test for avoidance behavior by rapidly moving away from strange objects (refs. 79,82). This behavior appears in the very early stage of development of galinaceous chicks and is almost certainly essential to their survival in the wild. Seven-day-old coturnix chicks were given Aroclor 1254 in their diet for 8 days and in untreated food for 6 more days. Avoidance behavior was measured daily, and a group avoidance response was significantly suppressed by chlordane,

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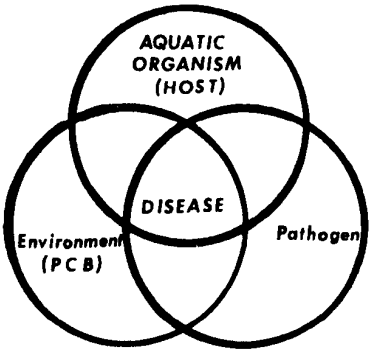


Figure 4. Relationship between environmental stress imposed by PCB and pesticides, susceptible host, and the disease organism as suggested by Sniezko (ref. 70).

dieldrin, and Aroclor 1254, although DDE had no apparent effect. Whereas the behavior of untreated birds returned to normal after 2 days on untreated feed, the response of chicks that were on dieldrin- and chlordane-treated feed improved somewhat during the 6 days of untreated food. However, the response of birds treated with Aroclor 1254 showed no improvement during the period.

Investigative monitoring for pesticide and PCB residues indicated that the highest residues occur in or near urban and industrialized areas. If these data are transferable to many other species of birds, we would expect to see the avoidance reaction suppressed, mortalities would increase from accidents, and predation would be maximized through the encroachment of man on the habitat of the species. The causes of mortality were subjected to autopsy study plus chemical analysis in studies conducted from 1969 through 1970 on 39 bald eagles found dead in various areas in the United States (refs. 42, 57, 79-82). The median residues of DDE in carcuses were 7 ppm in 1969 and 19 ppm in 1970, and mean residues of dieldrin were 0.4 ppm in 1969 and 0.7 ppm in 1970. Six of the eagles had dieldrin residues in the brain of 4.6 to 11 ppm, which is within the lethal range. One eagle contained 385 ppm of DDE and 235 ppm of PCB. In summary, environmental poisons were credited with 18 percent of the deaths, illegal shooting with 46 percent, accidents with 15 percent, natural disease with 8 percent, and miscellaneous and unknown causes with the remaining 13 percent.

In a survey to determine the degree of eggshell thinning and the extent of chemical contamination in aquatic birds in the Texas Coast area, eggs from 21 species were collected in 1971 (refs. 52, 78-80). Chemical analysis completed for 14 species showed that DDT in metabolites existed in all samples, whereas dieldrin residues were found in the Texas Rice Belt and the highest PCB residues were near urban and industrial areas again (ref. 79). The most noticably high PCB residues were found in some eggs of inland feeding species, including: double-crested cormorant (*Phalacrocorax auritus*), which contained 32 ppm; Caspian terns (*Hydroprogne caspia*), 16.5 ppm; and Foster terns (*Sterna forsteri*), 12.5 ppm (ref. 80). However, PCB were present in only 66 percent of the 158 eggs that were analyzed in another study conducted on ospreys (*Pandion haliaetus*) in the Gulf of California. During 1971, the residues of DDT were found to be generally low compared to those in osprey populations showing poor reproductive success in other parts of the range. PCB residues were detected in four of eight eggs and when present were higher than DDE residues; however, eggshell thinning was minor (averaging -3 percent), as expected from their low residues.

An experimental study on the absorption and metabolism of the PCB mixture Aroclor 1254 showed that all the components in the mixture were absorbed in essentially equal proportions from encapsulated dosages (ref. 81). The dietary dosage for quail of 300 ppm of Aroclor 1254 for 14 days followed by 14 or 42 days of untreated food showed that the various components of the Aroclor were excreted at greatly different rates. At the end of the 42 days of clean food, the PCB components changed entirely from the proportions of compounds that were originally fed to birds. Changes in patterns of gas chromatographic peaks that were detected militate against the dependable measurement of the quantities of PCB by gas chromatographic methods that can be related to the environmental or diet intake of the birds.

In another study, the combined effects of p,p'-DDE, and PCB were measured in mallard ducks. The ducks were fed diets containing 40 ppm of each chemical in the diet for 2 months prior to breeding and throughout the reproductive season (ref. 81). Average decreases in eggshell thickness were almost identical between the two compounds, with -17.5 percent for DDT only and -17.0 percent for DDE plus PCB in the diet (refs. 38, 79-80). No eggshell thinning occurred in birds fed PCB alone or in untreated diets except for a

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slight decrease in shell thickness near the end of the egg laying period. However, the birds fed the DDE-plus-PCB diet layed fewer eggs and egg production dropped off significantly 8 weeks before that of birds that were receiving other treatments. Egg breakage and egg eating were also highest in the DDE-plus-PCB groups and the combination of the chemicals seemed to increase the amount of egg loss through some effect—possibly on behavior. Therefore, overall production problems were most severe when PCB were fed in addition to the DDE.

In the Upper Great Lake States, 9 of 13 species of fish-eating birds were investigated in 1969-1970, and were found to sustain statistically significant decreases in eggshell thickness since 1946 (ref. 82). Maximum changes in thickness index occurred in great blue herons (-25 percent), red breasted mergansers (-23 percent), common mergansers (-15 percent) and double-crested cormorants (-15 percent). Great blue heron eggs taken from Louisiana generally displayed smaller changes since 1946 than herons in the Midwest. On a lipid basis, mean PCB and DDE residue levels exceeded 100 ppm in seven of these species in the Lake States and one of seven species in Louisiana. The average DDE:PCB ratios in the two regions were 1.26:1 and 3.9:1 respectively. These ratios contrast with some around 0.3:1 previously reported in oceanic birds in the Bay of Fundy and with ratios of roughly 6:1 and 10:1 that were reported by others on the Pacific Coast. The relationship between shell thinning and DDE content of eggs was apparent for most species, but for herons DDE was the only compound correlated (ref. 52).

Partial correlation carried out on a somewhat questionable family basis showed that PCB also correlated with shell thinning in mergansers, in a study of chlorobiphenyl poisoning with formation of myelin figures in both mouse and monkey liver (ref. 49). Mice were subjected to daily dosages of .001 ml for 12-26 weeks and cornification of smooth surface membranes of the cytoplasmic reticulum was observed (ref. 50). This was also accompanied by a displacement of rough surface membranes and endoplasmic proliferation and an increase in microbodies, lyosomes, and lipid content. Similar observations were made in the squirrel monkey from dosages of 320 mg given over 46 days. Liver enlargement was noted but the main cause of death was attributed to penumonia or diarrhea (ref. 49). A similar experiment was run on the cynomolgus monkey, which received 64.1 mg in 40 days to 348 mg in 239 days with very similar results (ref. 49). Sublethal effects of hepatic enzyme induction were correlated with the breakdown of estradiol and the increase in cytoplasmic RNA in kestrels fed 0.5 to 5.0 ppm of Aroclors 1254 or 1262 for 5 months (ref. 53).

The effects of polychlorinated biphenyls and organochlorine pesticides appear to be manifested in the alteration of enzyme and hormone biochemistry. In unpublished work by Street and his coworkers, Vos reported that in inductions of hepatic microsomal enzymes, the sleeping time of experimental animals was decreased after treatment with hexoberbitol. An enhancement of in vitro rates of aniline hydroxylation and para-nitroanisole demethylations occurred in direct proportion to an increase to chlorine content of different PCB isomers in Aroclors 1221 through 1268 (ref. 86). PCB preparation of Aroclor 1221 and Aroclor 1254 at 1 and 10 mg/kg for 28 days in pregnant rabbits resulted in liver enlargement with increased activity of drug metabolizing enzymes aniline hydroxylase and aminopyrine n-demethylase at the rate of 10 mg/kg of Aroclor 1254, while no effects could be measured at the other levels and with the other levels and with the other Aroclors (ref. 86).

Vos conducted an extensive review of toxicity of PCB for mammals and birds (ref. 86). He concluded that PCB's have several sublethal effects, such as microsomal enzyme induction, porphyrogenic action, estrogenic activity, and immunosuppression. Aroclor 1254 administered to rabbits during the first 28 days of gestation had embryo-toxic effects at levels of 12.5, 25, and 50 mg/kg of body weight, which demonstrates the direct effects on microsomal enzyme activity in pregnant rabbits (ref. 77). In previously cited work by McLaughlin (ref. 44), edema and beak deformities in chicken embryos have been described after yoke sac-injection of 10 and 25 mg of Aroclor 1242 resulted in a 95 and 100 percent embryonic mortality respectively. PCB have also been found to cause neural pathology in rats when administered at a rate of 0.3 to 0.5 ml/kg per day for 14 or 21 days. The pathology is characterized by impairment of motor function and decreased motor velocity with loss of large nerve fibers (ref. 76).

A high dose of Aroclor was required before liver necrosis developed in chickens (ref. 88). Moderate dosages in rabbits produce mottled liver with subacute yellow atrophy and fatty degeneration with marked necrosis at dosage rates of 0.3 and 0.6 g/day, but 0.9 g/day caused death before liver pathology developed (refs. 86,87). Miller (1944) observed 100 percent mortality between 17 and 98 days with similar skin applications of 34.5 mg during an 11-day period caused 100 percent mortality within 28 days while on treatment (ref. 41). Central atrophy of liver cells occurred, with perinuclear basophilic granulation and focal necrosis occurring in a few of the animals.

Nichizumi (1970) utilized light and electron microscopy to confirm the formation of so-called myelin figures in mouse liver associated with a minimum effec-

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tive dose of 5 mg/kg of a 48% chlorine PCB (refs. 73,88). Japanese quail demonstrated porphyrogenic action when dosed with PCB for 7 days at dosage rates of 1 to 100 mg/kg of Aroclor 1260 (ref. 86). Nichizumi measured the formation of aminoevulinic acid by liver mitochondria; significant increases of greater than 10.5 to 120 m/moles ALA per gram of liver per hour were found. PCB concentrations in the liver ranged from 1.4 to 478 ppm between the 1 mg/kg and 100 mg/kg dosage rates. The most marked effect was noted at the 100 mg/kg dosage rate, in which microscopic tissue fluorescence was seen in three out of five cases and two out of five cases respectively.

Domestic chickens demonstrate an acute susceptibility to Aroclor intoxification; liver enlargement occurs at dosages ranging from 100-1,000 ppm of Aroclor 1242 and mortality occurs at rates above 200 ppm (ref. 88). However, Aroclor 1258 causes mortality at dosages above 100 ppm and partial mortalitites at dosages above 30 ppm when fed in a diet for 4 to 5 weeks. One hundred percent mortality occurs in chickens fed Aroclor 1254 above 250 ppm. Pathologically, clinical observations demonstrate that general edema occurs at all toxic dosages (refs. 7, 10, 35, 43, 55, 56). Internal haemorrhaging and tubular dilatation in kidneys are accompanied by enlargement of the kidneys, in addition to defeathering and dermatitis (refs. 86,88). Depression and secondary sexual characteristics occurred at levels as low as 30 ppm of Aroclor 1248 (ref. 7).

Sublethal effects caused by enzyme induction and increased steroid metabolism have been demonstrated in pigeons. American kestrels fed Aroclor 1254 and 1262 at levels of 0.5 and 5 ppm for 5 months affected hormone metabolism (refs. 53, 66, 67). In an interaction of polychlorinated biphenyls and duck hepatitis virus, 10-day-old ducklings fed Aroclor 1254 at levels of 25, 50 and 100 ppm had significantly higher mortality than those birds not exposed to PCB (ref. 13). Vos and Koeman (1970) found direct effects on the lymphoid system in chickens (ref. 88). Peakall and Lincer (1972) observed embryonic mortality and chromosomal aberrations in ring doves fed Aroclor 1254 at 10 ppm over two generations (ref. 54). They found that 13 of 17 PCB-treated embryos had aberration rates exceeding the mean control. Results thus indicate possible clastogenic (i.e., chromosome-breaking) action of PCB in dove embryos. Eggshell thinning was not observed in either the first or second generation. Dahlgren et al. observed that pheasant fertility and eggshell thickness were not affected by PCB exposure (ref. 10). The chief effects on reproduction occurred through PCB taken in by the hen but not by the cock. In laying pheasant hens, the major affects were observed in egg production, hatchability, and viability of the embryo. There also was a subtle effect on behavior, as the visual cliff and ability of offspring to avoid hand capture was adversely affected.

Among the most profound demonstrations of the adverse affects of PCB was the inhibition of mink reproduction that was caused by feeding them coho salmon containing PCB residues (refs. 2, 62, 63).

CONCLUSIONS

Polychlorinated biphenyls are highly persistent environmental contaminants that bioaccumulate in food chains and produce direct acute and chronic effects as well as subtle impact on the growth, reproduction, behavior, and health of fish, birds, and mammals. Freshwater fish are particularly susceptible to chronic effects since PCB residues concentrate at high levels that are known to elicit serious toxicological effects. Fish-eating birds and mammals are thus subject to hazardous levels of residues. However, the actual mode of action and ecological effects are poorly understood, as is the long-term impact of PCB on animal populations.

ACKNOWLEDGMENTS

The staff of the Fish-Pesticide Research Laboratory under the direction of Dr. Richard A. Schoettger contributed the major portion of the information on effects of PCB on aquatic organisms. The detailed *Quarterly and Annual Reports of the Research Divisions of the Bureau of Sport Fisheries and Wildlife* during 1969-1972 were especially helpful in addition to the publications and unpublished manuscripts from which the tables and figures were developed. The technical assistance of Drs. David L. Stalling, Paul M. Mehrle, Foster L. Mayer, and Mr. Jerry R. Longcore and Mrs. Laura Crane was particularly helpful.

SCIENTIFIC NAMES

Cutthroat trout (*Salmo clarki*); rainbow trout (*Salmo gairdneri*); Atlantic salmon (*Salmo salar*); coho salmon (*Oncorhynchus kisutch*); lake trout (*Salvelinus namaycush*); Japanese or coturnix quail (*Coturnix coturnix*); bald eagle (*Haliaeetus leucocephalus*); double-crested cormorant (*Phalacrocorax auritus*); caspian tern (*Hydroprogne caspia*); Fosters tern (*Sterna fosteri*); osprey (*Pandion haliaetus*); mallard duck (*Anus platyrhynchos*); great blue herons (*Ardeo herodias*); red breasted merganser (*Mergus serratus*); common merganser (*Mergus merganser*); American kestrel (*Falco sparverius*); ring dove (*Steptopelia risoria*); pigeon (*Columba livia*); pheasant (*Phasianus colchicus*); squirrel monkey (*Saimiri*

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sclureus); cynomolgus monkey (*Macaca sp.*); mink (*Mustela vison*).

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PCB's: EFFECTS ON AND ACCUMULATION BY ESTUARINE ORGANISMS

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Abstract

Effects of PCB's on and accumulation by estuarine organisms were studied in laboratory bioassays. Aroclors 1016, 1242, and 1254 were acutely toxic to certain estuarine organisms at concentrations greater than 10 µg/l, but these bioassays underestimated toxicities of PCB's, as shown by data from exposures that lasted longer than 2 weeks. Concentrations that were lethal to selected invertebrates and fishes in chronic exposures ranged from 0.1 to 5 µg/l. Reproduction of sheepshead minnows was impaired by concentrations of Aroclor 1254 in their eggs >5 µg/l, but this was not observed in eggs that contained up to 77 µg/g of Aroclor 1016. Bioaccumulation of PCB's in estuarine organisms generally exceeded 10^4 times the concentration in water in laboratory studies and 10^5 times in the estuary.

Following the discovery of PCB's in Escambia Bay, Florida, in 1969 (ref. 1), the Environmental Research Laboratory at Gulf Breeze, Florida, began studies with PCB's to determine their effects on and bioaccumulation by estuarine organisms. Estuarine organisms studied included bacteria (ref. 2); protozoans (ref. 3,4); oysters (refs. 5,6); shrimp (refs. 7,8,9,10,12,13); fishes (refs. 14,15,16,17,18,19); and communities of benthic organisms (ref. 20). The following discussion is confined to research on Aroclor® 1016, 1242, 1254 because these PCB's are presently produced in the greatest quantities.

Aroclors 1016, 1242, and 1254 are acutely toxic to certain estuarine organisms. The 48- or 96-hour LC50's, in µg/l, range from 9 to 32 for penaeid shrimp, 12 to 16 for grass shrimp, >100 for pinfish and EC50 (reduction of shell growth for oysters) from 10 to 32 µg/l (refs. 1,15,21).

Laboratory bioassays lasting longer than 2 weeks demonstrate that acute bioassays underestimate the toxicities of Aroclors 1016 and 1254. Aroclor 1254 is toxic to commercially valuable shrimps (*Penaeus* spp.) and grass shrimp (*Palaemonetes pugio*) at concentrations of 1 µg/l (refs. 8,11). Exposed shrimp are particularly sensitive to salinity stress (ref. 11) and possibly to viral disease (ref. 13). Aroclor 1254 decreases growth rates of oysters (*Crassostrea virginica*), at 4 µg/l (ref. 5). At concentrations of about 5 µg/l, Aroclor 1254 is lethal to juvenile fishes: spot (*Leiostomus xanthurus*), pinfish (*Lagodon rhomboides*), and sheepshead minnow (*Cyprinodon variegatus*) (refs. 14,19). At Aroclor 1254 concentrations of 0.1 µg/l, some sheepshead minnow fry die (ref. 19). Aroclor 1016, at 15 µg/l, was lethal to pinfish and sheepshead minnows; susceptibilities of fry, juvenile, and adult sheepshead minnows were similar (refs. 15,18).

Aroclor 1254 effects reproduction of the sheepshead minnow, an estuarine fish (ref. 18). Adult fish exposed for 4 weeks to 0.1 µg/l appeared to be unaffected, but when eggs from these fish were fertilized and placed in PCB-free water, the survival of fry was diminished. Mortality was observed also in fry from eggs that contained greater than 5 µg/g of the PCB and increased as PCB content of the eggs increased. Reproductive success of Atlantic salmon (ref. 22) and striped bass (ref. 23) may also be affected by PCB's in their eggs. Adult sheepshead minnows that had been exposed for 4 weeks to from 0.3 to 3 µg/l of Aroclor 1016 produced eggs that contained from 3 to 77 µg/g of this PCB, yet fry that hatched from these eggs were apparently unaffected (ref. 18).

Estuarine organisms accumulate PCB's from water. Maximum concentration factors (concentration in animals divided by concentration in water) in laboratory exposures of various species to Aroclor 1254 were: oysters, 101,000 (ref. 5) to 165,000 (ref. 8); shrimp, 26,000 (ref. 9); and fishes, 37,000 (refs. 14,19). The maximum concentration factor determined from exposures of fishes to Aroclor 1016 was 34,000 (refs. 15,17). Fishes exposed continuously to Aroclors 1016 and 1254 accumulated both Aroclors in increasing concentrations for about 4 weeks; thereafter, the quantity stabilized (refs. 14,15). Maximum concentrations were found in the liver, gills, and skin of the fish, but the lowest concentration found in fishes and shrimps was in the muscle (refs. 14,15,8). Bioconcentration factors calculated from data from Escambia Bay (refs. 1,10) were greater than 230,000 for shrimp, 870,000 for fishes, and greater than 100,000 for oysters, indicating that laboratory data can underestimate bioconcentration potentials of Aroclor 1254.

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SUMMARY OF RECENT INFORMATION REGARDING EFFECTS OF PCB'S ON FRESHWATER ORGANISMS

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Abstract

Polychlorinated biphenyls (PCB's) are toxic to freshwater organisms at concentrations below 5 µg/l (ppb), and newly hatched fish and small insects and crustaceans with short life cycles are most sensitive. Aroclor 1254 at 0.45 µg/l produced a 50 percent decrease in midge reproduction; 1.3 µg/l caused a 50 percent reduction in Daphnia reproduction; and 1.8 µg/l produced 50 percent reduction in fathead minnows. The indirect toxicity of PCB's to predators through accumulation of PCB's in tissues of food organisms causes death. Fish can accumulate 200,000 times more PCB's in their flesh than can be held in the surrounding water. The concentration of Aroclor 1260 in the water resulting in a tissue residue of 0.5 µg/l is approximately 0.002 µg/l. Aroclor 1016 and 1242 have been shown to have similar toxicity to freshwater organisms.

The Environmental Protection Agency proposed water quality criterion for PCB's is as follows: "Maximum acceptable concentration of PCB's in water is 0.001 µg/l."

Polychlorinated biphenyls (PCB's) are toxic to freshwater fish and other aquatic organisms at very low levels and direct toxic effects are evident within a few hours, but much greater harm is caused by longer exposure at lower concentrations. PCB levels that kill fish, crustacea, and aquatic insects are similar to those of DDT and several other pesticides. Environmental occurrence of these materials is increasing and there is mounting evidence that they are causing widespread harm in the freshwater aquatic environment.

One of the significant accomplishments since the discovery of the toxicity of PCB's to freshwater organisms was the synthesis of known data and their publication in the "Blue Book," or *Water Quality Criteria—1972*, sponsored by the U.S. Environmental Protection Agency and prepared by the National Academy of Sciences, National Academy of Engineering Committee on Water Quality Criteria (ref. 1). It was recommended in this report that aquatic life should be protected where the maximum concentration of total PCB in unfiltered water does not exceed 0.002 µg/l at any time or place, and the residues in the general body tissues of any aquatic organism do not exceed 0.5 µg/g.

The Environmental Protection Agency (ref. 2) also proposed water quality criteria for PCB's as follows: "Maximum acceptable concentrations of PCB's in water are 0.002 µg/l, and maximum acceptable levels of residues in general body tissues of any aquatic organism are 0.5 µg/l"; following the proposed criteria recommended by the National Academy "Blue Book." However, these criteria have not been promulgated. Recently, the Environmental Protection Agency (ref. 3), using the latest information available, has proposed 0.001 µg/l PCB as a maximum permissible concentration in freshwater, but has proposed no tissue level criterion.

The purpose of this paper is to present a summary of recent information dealing with the toxicity of polychlorinated biphenyl compounds to freshwater fish, crustaceans, and aquatic insects, and to present important recent information on the bioconcentration of PCB's in fish tissues and its effect on consumer organisms.

Fish

A significant amount of new information dealing with the toxicity of PCB's to fish has been collected during the last 3 years. Acute and longer term studies have been conducted with a variety of freshwater sport and forage fish species. Tests with fathead minnows at the National Water Quality Laboratory, Duluth, Minnesota, (ref. 4) have shown that Aroclor 1242, A-1248, and A-1254 are toxic below 5 µg/l. Ninety-six-hour LC_{50} values (that concentration which kills half the fish in 96 hours) for newly hatched fathead minnows were 15 µg/l for Aroclor 1242 and 7.7 µg/l for A-1254. Fifty percent died at 8.7 µg/l A-1248 after 30 days. After 60 days, half were dead at 8.8 µg/l A-1242 and 4.6 µg/l A-1254 (table 1).

Two 9-month continuous-flow bioassay tests were conducted (ref. 4) with the fathead minnow to determine the effects of Aroclor 1242 and A-1254 on survival and reproduction (table 2). Concentrations of Aroclor 1242 above 10 µg/l were lethal to newly hatched fry and all minnows were dead after 96 hours in 51 µg/l A-1242; none survived to the end of the test at 15 µg/l. Fry survived when reared in the same concentrations at which their parents lived and spawned; however growth of newly hatched fish was retarded prior to their death

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Table 1. Acute toxicity of Aroclor 1242, 1248, and 1254 to fathead minnows, Gammarus and Daphnia, in continous-flow tests

	Test temp. (C)	96-hr LC_{50}[a] (µg/l)			60-day LC_{50} (µg/l)		
		1242	1248	1254	1242	1248	1254
Fathead minnow, newly hatched	24°	15	*[b]	7.7	8.8	8.7[c]	4.6
Gammarus	18°	73	29	--	8.7[c]	5.1[c]	--
Daphnia	18°	--	12.9	6.4	--	2.6[d]	1.8[d]

[a]Concentration where 50 percent died after 96 hours.

[b]No mortality after 96 hours.

[c]Thirty-day LC_{50}.

[d]Fourteen-day LC_{50}.

Table 2. Spawning and egg production of fathead minnows exposed to Aroclor 1242 and 1254

Aroclor concentration (µg/l)	Number of spawnings per female	Number of eggs per spawning	Number of eggs per female	Percent eggs hatched
Aroclor 1242				
51.0	0	0	0	0
15.0	0	0	0	0
5.4	2.5	30	151	81
2.9	3.9	63	283	38
0.9	1.3	28	35	84
0.0	4.6	90	442	53
Aroclor 1254				
15.0	0	0	0	0
4.6	0	0	0	0
1.8	1.0	63	106	79
0.5	5.2	105	556	63
0.2	3.0	64	221	55
0.0	2.4	104	253	75

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larvae of the mosquito *Culex tarsalis*, exposed to 1.5 µg/l Aroclor 1254, survived well and pupated, but many of the pupae were unable to metamorphose into the adult form. Control organisms pupated with success.

Bioconcentration

Bioconcentration, biological magnification, bioaccumulation, PCB or tissue residues, and residue dynamics, are some of the terms used to describe the intake of PCB's from food or direct water contact into the animal and accumulation in the tissues to concentrations many thousands of times those in the water or food they were exposed to. Polychlorinated biphenyls are much more soluble in fatty tissues (lipids) than water and are selectively concentrated in those tissues. Animals that feed on aquatic invertebrates or fish that have bioaccumulated significant tissue concentrations of PCB's are threatened even when the prey species are not adversely affected.

Exposure of fish to water containing PCB's demonstrates that they can bioconcentrate PCB's directly from the water, in addition to uptake in food, and in most cases direct uptake from water is more rapid and leads to much higher accumulation in the tissues. The bioconcentration factor (concentration in fish tissue/concentration in the water) for PCB's is essentially independent of the PCB concentration in the water. The bioconcentration factor (whole body) for adult male fathead minnows at 25° C is approximately 1.2×10^5 (120,000) for Aroclor 1248 and 2.7×10^5 (270,000) for Aroclor 1260. Female fathead minnows accumulated about twice as much PCB's as the male fatheads and the difference is largely

The residue of PCB's in the tissues of fathead minnows studied by DeFoe et al. (ref. 7) was directly proportional to the concentration of the toxicant in the water. The concentration of Aroclor 1248 in the water resulting in a tissue residue of 0.5 µg/g was approximately 0.004 µg/l. The concentration of Aroclor 1260 in the water resulting in a tissue residue of 0.5 µg/g was approximately 0.002 µg/l. They showed that the more highly chlorinated PCB mixtures are bioconcentrated to a greater extent in the lipids of the fish than the lower chlorinated compounds. They also showed that the PCB residues in the fish reached an apparent steady state after approximately 100 days, and when exposed fish were placed in untreated Lake Superior water they eliminated less than 18 percent of the body burden after 60 days.

Nebeker et al. (ref. 4) showed that the bioconcentration factor for Aroclor 1254 in fathead minnows ranged from 1.1×10^5 to 2.4×10^5 and that the bioconcentration factor for several PCB's in the fathead minnow ranged from about 0.3×10^5 to 2.7×10^5 with the more highly chlorinated PCB's accumulating to a greater extent. Adult fathead minnows accumulated up to 430 ppm A-1242 in whole body residues, with concentrations up to 270,000 times that of water, and up to 1,000 ppm of 1254, with concentrations up to 230,000 times that of the water (ref. 4), *Gammarus* accumulated over 500 ppm 1248 and 400 ppm 1242, with concentrations over 100,000 times that of the water (ref. 14).

Sodergren and Svensson (ref. 19) showed that the nymph of *Ephemera danica* accumulated Clophen A 50 to bioconcentration factors of 3×10^3. After 60 days of exposure to Aroclor 1242 and A-1248, the maximum bioconcentration factor in *Gammarus pseudolimnaeus* was approximately 3×10^4 and 5×10^4, respectively (ref. 14).

Sanders and Chandler (ref. 18) showed that the uptake and biological magnification of Aroclor 1254 by some aquatic invertebrates was very rapid. When *Daphnia magna* were exposed for 4 days to water containing 1.1 µg/l of A-1254, they accumulated total body concentrations 48,000 (4.8×10^4) times greater than those in water. The mosquito larvae *Culex tarsalis* exposed to water containing 1.5 µg/l A-1254 accumulated 19 µg/g of this compound within 24 hours, which represents a 12,600-fold magnification. Scuds, *Gammarus pseudolimnaeus*, exposed continuously to 1.6 µg/l A-1254, reached an equilibrium concentration in 14 days. Total body residues were 44 µg/g, a concentration 27,500 times that in the water.

Some evidence exists that the bioconcentration factors measured in the laboratory are essentially the same as those found in the river environment. Veith and Lee (refs. 20, 21) reported that goldfish in the lower Milwaukee River accumulated Aroclor 1248 approximately 0.7×10^5 to 2×10^5 times (depending on the lipid content) the estimated PCB concentration in the river water. The maximum concentration of Aroclor 1254 in Saginaw River shad was 165 µg/g whereas typical water concentrations averaged 1.1 µg/l, with a maximum of 2.9 µg/l (ref. 22). This indicates a bioconcentration of 0.6×10^5 to 1.5×10^5.

Studies resulting from the reproductive failure of mink fed Great Lakes fish led to the conclusion that it was PCB residues in the fish that caused the adverse effects. Ringer et al. (ref. 23) demonstrated that 10 µg/g of Aroclor 1254 in coho salmon produced 71 percent mortality in mink and that a mixture of 10 µg/g PCB's and 0.5 µg/g dieldrin in coho feed produced 100 percent mortality. No kits were born alive when the diet contained 5 µg/g or greater Aroclor alone. The "no effect" concentration of PCB's in the mink diet was estimated to be less than 1.0 µg/g.

Platonow and Karstad (ref. 24) presented similar

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at higher PCB concentrations. Reproduction occurred and good egg hatching took place at and below 5.4 µg/l A-1242. Eggs produced by control fish but maintained at the higher concentrations of 15 and 51 µg/l hatched with good success but none of the fry survived.

Aroclor 1254 was more toxic to the fathead minnow than A-1242 (ref. 4), as they did not survive and reproduce above 1.8 µg/l A-1254 (table 2). All fish in the long-term study were dead after 96 hours at 15 µg/l, and growth was delayed at 4.6 µg/l. Spawning occurred at 1.8 µg/l but was significantly less than at the lower concentrations. Egg hatchability and fry survival were good at and below 1.8 µg/l. Eggs produced by the control fish and maintained at 15 µg/l hatched readily, but all young fish were dead within 96 hours.

Growth of young fathead minnows was affected above 2.2 µg/l A-1248, and none survived above 5.1 µg/l after 30 days. Young flagfish did not survive at Aroclor 1248 concentrations above 5.1 µg/l and did not grow well above 2.2 µg/l (ref. 4).

Due to low PCB solubility, 96-hour LC_{50} values do not adequately reflect PCB toxicities to fish. For Aroclor 1221-1268, 96-hour LC_{50} values ranged from 1,170 to 60,000 µg/l for cutthroat trout. The acute oral toxicity of Aroclors 1242, 1248, 1254, and 1260 was greater than 1,500 mg/kg in rainbow trout. Intermittent-flow bioassays of A-1242, 1248, and 1254 to bluegills resulted in 16-day LC_{50} values of 64, 76, and 204 µg/l, respectively. Exposures to channel catfish gave 15-day LC_{50} values of 107, 127, and 741 µg/l for the same Aroclors. All LC_{50} values decreased significantly with longer exposure, indicating the much greater PCB hazard with increased exposure time (ref. 5). Direct water exposures appear to represent a greater hazard to fish than dietary exposures, but dietary sources are important as PCB's have a high affinity for sediments and readily enter the food chain.

Schoettger et al. (ref. 6) showed that the growth of lake trout was retarded by Aroclor 1248 in the diet for 3 months. Concentrations of 1.2, 3.8, and 12 µg/g decreased weight gain by 6, 10, and 28 percent, respectively, below that of controls. The growth of the high-exposure group (12 µg/g in diet) was only about half that of the controls after 6 months.

Fathead minnows were exposed to Aroclor 1248 and A-1260 (ref. 7), in flow-through bioassays to determine the acute (30-day) and chronic (240-day life cycle) effects on the fry and adult fathead minnows. Newly hatched young were the most sensitive lifestage to the exposure of PCB's. Total mortality occurred when newly hatched fatheads were exposed for 30 days to 8.5 µg/l A-1248. All fish exposed to 9.0 µg/1 A-1260 died after a 30-day exposure. The calculated 30-day TL_{50} for newly hatched fathead minnows (<8 hours old) was 4.7 µg/l for A-1248 and 3.3 µg/l for Aroclor 1260. PCB's did not measurably affect the ability of fathead minnows to reproduce at concentrations which were acutely toxic to the larvae. With reproduction occurring at and below 3 µg/l for A-1248 and at below 2.1 µg/l for A-1260, the 20 percent reduction in the standing crop in the second generation of minnows at concentrations as low as 0.4 µg/l is due to the mortality of the fathead larvae soon after hatching.

Aroclor 1016, a compound chemically similar to A-1242, has been introduced recently to replace PCB's of the Aroclor 1200 series in many applications. Work has been completed with this compound with freshwater organisms and what is available indicates that the toxicity of Aroclor 1016 is similar to that of Aroclor 1242.

Mayer (ref. 8) presents data on the toxicity of Aroclor 1016 to nine species of freshwater fishes. Bluegills had a 4-day LC_{50} of 260 µg/l A-1016, rainbow trout, 135 µg/l; Atlantic salmon, 134 µg/l; and yellow perch, 185 µg/l A-1016.

Johnson (ref. 9) presented data comparing the effects of A-1242 and A-1016 on rainbow trout and bluegill sunfish. Rainbow trout had a 10-day LC_{50} of 39 µg/l for A-1242 and 17-day LC_{50} of 49 µg/l for A-1016. Bluegills had a 15-day LC_{50} of 54 µg/l A-1242 and a 35-day LC_{50} of 43 µg/l for A-1016. They had 96-hour LC_{50} values of 125 µg/l A-1242 and 46 µg/l A-1016.

A test comparing the toxicity of Aroclor 1242 and Aroclor 1016 using fathead minnows was completed by Veith (ref. 10). There was no difference in the survival of fry after 96 hours between A-1242 and A-1016 at 28 µg/l; both had 50 percent mortality. In 30-day tests, 100 percent mortality of fry occurred at 42 µg/l A-1242 and 95 percent mortality occurred at 42 µg/l A-1016. Approximately 50 percent had died after 30 days at 25.5 µg/l A-1242 and 30.5 µg/l A-1016.

The effects of Aroclor 1242 on the guppy *Poecilia reticulata* were studied by Morgan (ref. 11) in static tests. The guppy had 100 percent mortality at 20 and 2 ppm, but only 25 percent mortality at 0.2 ppm after 7 days.

In preliminary investigations, Jensen, et al. (ref. 12) reported a possible relationship between PCB residues in salmon eggs and egg mortality in Sweden. When residues in groups of eggs ranged from 0.4 to 1.9 µg/g on a whole-weight basis (7.7 to 34 µg/g on a fat basis), related mortalities ranged from 16 to 100 percent.

No adverse effects were observed on survival, growth, and reproduction of brook trout exposed for 71 weeks to 0.94 µg/1 and lower concentrations of the PCB

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Aroclor 1254. Survival and growth to 90 days of alevin-juveniles from exposed parents were also unaffected. PCB concentrations in the brook trout were directly proportional to the water exposure concentration. PCB tissue concentrations appeared to have reached a steady state by the first sampling following 14 weeks of exposure. PCB residues (wet tissue basis) in chronically exposed fish were approximately 2 µg/g in the fillet and 9 µg/g in the "whole body" (minus one fillet and the gonads) at the highest water concentration, 0.94 µg/l. The higher residue in the "whole body" compared to the corresponding fillet was due to the higher fat content of the former (ref. 13).

Thyroid stimulation in coho salmon ranged from a 52 percent increase in fish fed 0.48 µg/g Aroclor 1254 of food to a 119 percent increase in the high-treatment group (480 µg/g). The lowest detectable stimulation of thyroid activity occurred in salmon dosed with 1/1000th that caused mortality. In channel catfish, 2.4 µg/g and 24 µg/g A-1254 in food caused significant increases in thyroid activity when compared to control fish (ref 15).

Crustacea

Freshwater crustaceans are very sensitive to low concentrations of PCB's. Because of their generally short life cycles they will be eliminated from PCB-contaminated waters quite rapidly, causing not only their loss from aquatic systems but impairment of fish populations because of a decrease in fish food organisms.

To assess the danger of PCB compounds to fish food organisms, bioassays were conducted with *Daphnia magna* (ref. 14) using the eight commercially available PCB's, Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268 (table 3). Aroclor 1248 was the most toxic to *Daphnia magna* of the eight Aroclors tested in static tests; the 3-week LC_{50} was 25 µg/l. Aroclor 1232, 1242, and 1254 had similar toxicity values to A-1248. In continuous-flow Daphnia tests, Aroclor 1254 was the most toxic, with a 3-week LC_{50} of 1.3 µg/l. No reproduction occurred at 3.8 µg/l A-1254, and no adults survived 3.6 µg/l. The Aroclors were much more toxic in continuous-flow conditions due to continual replacement of fresh PCB. Almost one-third of the PCB added to the test water was lost to the air, with some lost to bacteria, algae, waste materials, test container surfaces, and test animal tissues. The difference between static and continuous-flow results (table 3) illustrates how critical test methodology can be for certain chemicals.

The freshwater scud *Gammarus pseudolimnaeus* was also studied by Nebeker and Puglisi (ref. 14) in continuous-flow test systems to determine the effects of A-1242 and A-1248 on survival and reproduction. Ninety-six-hour LC_{50} values were 73 µg/l for A-1242 and 29 µg/l for A-1248. Fifty percent were surviving after 60 days at 8.7 µg/l A-1242 and 5.1 µg/l A-1248. No live animals remained after 2 months at 26 µg/l A-1242 or 18 µg/l A-1248 (table 4). Good reproduction occurred at and below 2.8 µg/l A-1242 and 2.2 µg/l A-1248.

Table 3. Toxicity of eight Aroclors to *Daphnia magna* in static and continous flow bioassays

Aroclor (PCB)	3-week LC_{50}[a] (µg/l)	50 percent reproductive[b] impairment (µg/l)
Continuous-flow test conditions		
1248	2.6	2.1
1254	1.8[c]	1.1
1254	1.3	1.3
Static test conditions		
1221	180	125
1232	72	66
1242	67	63
1248	25	24
1254	31	28
1260	36	33
1262	43	41
1268	253	206

[a] Concentration where 50 percent died.

[b] Fifty percent loss of young.

[c] Two-week LC_{50}.

Static and flow-through toxicity tests with aquatic invertebrates (ref. 5) indicate that they are generally more susceptible to acute toxic effects of PCB's than fish. *Gammarus faciatus* in flow-through tests had a 96-hour LC_{50} of 10 µg/l A-1242 and a 10-day LC_{50} of 5 µg/l A-1242. The glass shrimp *Palaemonetes kadiakensis* was very sensitive to A-1254, with a 7 day LC_{50} of 3 µg/l.

In static tests with the amphipod *Gammarus pseudolimnaeus*, Meyer et al. (ref. 15) found that of four PCB homologs tested, the trichlorobiphenyl was slightly more toxic than the di-, penta-, and hexachlorobiphenyls. The trichlorobiphenyl had a 96-hour LC_{50} of 100 µg/l, two dichlorobiphenyls tested had 96-hour LC_{50} values of 100 and 120 µg/l, the hexa- and penta-homologs had 96-hour LC_{50} values of 150 and 210 µg/l respectively.

The crayfish *Orconectes nais* was reported by Stalling and Mayer (ref. 5) to have a 7-day LC_{50} of 30 µg/l for Aroclor 1242 and a 7-day LC_{50} of 80 µg/l for A-1254.

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Table 4. Survival and reproduction of *Gammarus pseudolimnaeus* after 2 months exposure to Aroclor 1242 and 1248

Aroclor concentration (µg/l)	Survival of adults (percent)	Young per surviving adult
Aroclor 1242		
234.0	0	0
81.0	0	0
26.0	0	0
8.7	52	0
2.8	77	4
0.0	48	7
Aroclor 1248		
18.0	0	0
5.1	53	7
2.2	73	22
0.8	71	20
0.2	73	11
0.0	64	11

Daphnia pulex were quite sensitive to Aroclor 1242 (ref. 11). Young *Daphnia pulex* died at levels as low as 0.02 ppm in 4-day static tests. The toxicity of Aroclor 1242 and DDT to the ostracod *Cypidopsis vidua* appeared to be approximately the same in static tests. At 20 ppm, there were no survivors out of 20 ostracods in the Aroclor cultures, and some mortality occurred at 2 ppm.

Studies by Moorman and Biesinger (ref. 16) comparing the lethality of Aroclor 1016 and Aroclor 1242 to *Daphnia magna* have shown similar toxicity for both compounds in static tests. No young survived after 3 weeks at 125 µg/l A-1016 or A-1242. At 100 µg/l, 80 percent of the young (percent of control) survived after 3 weeks in A-1016 and 19 percent of the young survived A-1242. Good survival occurred at 50 µg/l for both compounds.

A study of the toxicity of isomers of Aroclor 1242 and 1254 to *Daphnia magna* by Biesinger (ref. 17) showed significant reproductive impairment in static tests at 50 µg/l for trichloro- and tetrachlorobiphenyls, indicating that there was little difference in the toxicity of the tri- and tetra- chlorobiphenyl compounds.

Aquatic Insects

Aquatic insects also appear to be very sensitive to longer exposures of PCB's, though response is variable when exposed to brief, or acute, concentrations. The midge *Tanytarsus dissimilis* was tested through its full life cycle (ref. 14) and found to be affected at levels below 1 µg/l. Survival and growth of the midge, when tested with Aroclor 1254, were excellent in control chambers but were reduced by 50 percent at the lowest test concentration of 0.45 µg/l (table 5). At 1.2 µg/l, the numbers of larval cases were reduced to 35 percent of the control, and the numbers of pupal cases were reduced to 18 percent of the control. No adult emergence occurred above 3.5 µg/l, and abundant adult emergence did not occur above 3 µg/l, even though larvae were present. No pupal cases were constructed at 9 µg/l and no larval cases were formed at 33 µg/l A-1254. The calculated 3-week LC_{50} for A-1254 (50-percent reduction based on control as 100 percent) was 0.65 µg/l for larvae and 0.45 µg/l for pupae. Adult midges emerged at concentrations up to 9 µg/l Aroclor 1248. Larvae were present at 18 µg/l, but adult emergence did not occur. Abundant emergence did not occur above 5.1 µg/l (ref. 12).

Mayer et al. (ref. 15) report some acute data for other aquatic insects. Dragonfly and damselfly nymphs, *Macromia sp.* and *Ischnura verticalis*, were tolerant to Aroclor 1242 and A-1254 in short-term static tests. The dragonfly had a 7-day LC_{50} of 800 µg/l for Aroclor 1242 and 1,000 µg/l for A-1254. The damselfly, tested under continuous-flow conditions, had a 4-day LC_{50} of 400 µg/l for A-1242 and 200 µg/l for A-1254.

Studies by Sanders and Chandler (ref. 18) have shown that larvae of the stonefly *Pteronarcys dorsata*, the dobsonfly *Corydalus cornutus*, and the phantom midge *Chaoborus punctipennis* survived concentrations of up to 2.8 µg/l A-1254 for up to 21 days without significant mortality. However, in similar experiments,

Table 5. Effect of Aroclor 1254 on the growth and survival of the midge *Tanytarsus dissimilis*

Aroclor concentration (µg/l)	Number of mature larval cases (percent of control)	Number of pupal cases (percent of control)
33.0	0	0
9.0	0.2	0
3.5	7	7
1.2	35	18
0.4	52	55
0.0 (control)	100	100

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evidence for mink fed diets enriched with Aroclor 1254. The data show that 0.64 μg/g of PCB in the rations resulted in only 1 of 12 mink producing 3 kits, all of which died on the first day.

Research with nonhuman primates indicates that the toxicity of PCB residues to consumers are not limited to mink. Barsotti and Allen (ref. 25) and Allen et al. (ref. 26) have shown that PCB enriched diets were toxic to primates. Female primates fed 2.5 μg/g Aroclor 1254 in their diet had periorbital edema, acneform lesions on face and neck, and substantially reduced fertility after 2 months.

SUMMARY

There is ample evidence that PCB's are toxic to freshwater organisms at very low concentrations. Newly hatched fish and small insects and crustaceans with short life cycles appear to be the most sensitive life forms. In larger animals or those with longer life cycles, toxic effects are delayed and acute or short-term testing does not adequately reflect the chronic or longer term effects that PCB's have on the test animals. Concentrations of polychlorinated biphenyls commonly found in contaminated waterways today, such as Aroclor 1242 and 1254, are directly toxic to fish and aquatic invertebrates in the range of 0.1 to 10.0 μg/l. Aroclor 1016 and 1242 have been shown to have similar toxicity to freshwater organisms. The indirect toxicity of PCB's to predators through accumulation of PCB's in tissues of food organisms may cause deaths from water concentrations that do not cause direct lethality.

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DISTRIBUTION AND EXCRETION OF [^{14}C]-2,4,5,2',5'-PENTACHLOROBIPHENYL IN THE LOBSTER (*Homarus americanus*) AND THE DOGFISH SHARK (*Squalus acanthias*)*

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Abstract

Significant ambient concentrations of polychlorinated biphenyl (PCB) residues were present in lobster hepatopancreas (1.57 ppm wet weight) and dogfish shark liver (1.87 ppm wet weight). PCB concentrations in muscle were only 10-20 percent of hepatic values. After injection of [^{14}C]-2,4,5,2',5'-pentachlorobiphenyl (^{14}C-2,4,5,2',5'-PCB) (0.2 mg/kg) into the pericardial sinus of the lobster, the hepatopancreas contained more than 90 percent of the recovered radioactivity at all times investigated (24 hr-8 wk). Virtually all of the hepatopancreas radioactivity was unaltered 2,4,5,2',5'-PCB. The radioactivity was very persistent in hepatopancreas, female egg mass, stomach, gill, and intestine of lobsters ($t_{½}$ > 3 wk). Following intravascular administration of ^{14}C-2,4,5,2',5'-PCB (0.03 mg/kg) to dogfish, almost all of the dosed radioactivity was found in liver at each time studied (6 hr-12 days). Most of the benzene extractable material was also unaltered 2,4,5,2',5'-PCB in each instance. Small amounts of radioactivity were excreted in bile but only traces in urine. Microsomes from lobster hepatopancreas had no detectable aniline hydroxylase, benzphetamine demethylase, 7-ethoxycoumarin O-deethylase, or benzpyrene hydroxylase activity although dogfish shark liver microsomes had low activity with each substrate tested. The persistence of ^{14}C-2,4,5,2',5'-PCB in the two marine species is probably related to the low hepatic microsomal mixed-function oxidase activities observed and the high lipid content of lobster hepatopancreas and dogfish shark liver.

INTRODUCTION

The polychlorinated biphenyls (PCB's) possess high chemical and thermal stabilities and have been used extensively in the United States for certain industrial applications during the past 45 years (ref. 1). PCB's were first recognized as environmental contaminants in 1966 (ref. 2). Subsequently, the widespread occurrence of PCB residues was verified in wildlife species (ref. 3) (both terrestrial and marine) all over the world, in human adipose tissue (ref. 4), in waters of the North Atlantic (ref. 5), and in marine species indigenous to Maine (ref. 6). The chemical stability and lipid solubility of certain PCB's contributes to their bioaccumulation.

Commercially available PCB's are complex mixtures of many different species with varying chlorine content. Aroclor 1254, for example, was resolved into more than 50 individual compounds, of which the pentachlorobiphenyl derivatives were the major constituents (ref. 7).

The present study describes the distribution and excretion of [^{14}C]-2,4,5,2',5'-pentachlorobiphenyl (^{14}C-2,4,5,2',6'-PCB) in the lobster (*Homarus americanus*) and the dogfish shark (*Squalus acanthias*) after parenteral administration. The in vivo data is correlated with in vitro microsomal mixed-function oxidase activities of lobster hepatopancreas and dogfish shark liver. A single, radiolabeled PCB isomer was used in the disposition experiments. Use of pure isomers has obvious advantages over similar experiments with commercial PCB mixtures. The isomer chosen is a constituent of Aroclor 1254, and should be representative of several Aroclor 1254 components. PCB residues were also determined in certain tissues of lobsters and dogfish sharks.

MATERIALS AND METHODS

Male or female lobsters (320-560 g) were purchased locally (Mt. Desert Island, Maine) and female dogfish sharks (3.0-5.1 kg) were caught by hook in Frenchman Bay, Maine. Both species were maintained in tanks equipped with circulating fresh seawater before and after treatment with ^{14}C-2,4,5,2',5'-PCB. The lobsters maintained for longer than 1 week after dosing were submerged in a lobster crate in Frenchman Bay. They were fed small pieces of mackerel or flounder three times a week when the traps were lifted to remove any dead or ill animals. Only lobsters that were alive and in apparent good health were dissected at the indicated times.

2,4,5,2',5'-pentachloro [^{14}C] biphenyl (^{14}C-2,4,5,2',5'-PCB), (10.98 mCi mmole) was obtained from Mallinckrodt Nuclear and was shown by thin-layer chromatographic analysis (TLC) to be greater than 98.5 percent radiochemically pure.

^{14}C-2,4,5,2',5'-PCB was injected into the pericardial

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sinus of lobsters (0.2 mg/kg, 1.4 X 10^7 dpm/kg) or into the caudal vessels of dogfish sharks (0.03 mg/kg, 2.2 X 10^6 kpm/kg) as a solution in Emulphor water (36-38 percent v/v).

Lobster plasma samples were withdrawn from the pericardial sinus just prior to sacrifice and placed in vials containing citrate. Dogfish blood samples (serially in disappearance studies or terminally in distribution studies) were taken from the caudal vessels and placed in heparinized vials. At various times after administration of ^{14}C-2,4,5,2',5'-PCB, animals were sacrificed and various tissues and fluids were removed from lobsters and dogfish. Duplicate aliquots of plasma, blood, bile, or cerebrospinal fluid (100 μl) or tissue (70-200 mg) were solubilized in 2 ml NCS (Amersham/Searle) by incubation at 50° C overnight. Liquid scintillation solvent (18 ml; 5.0 g PPO; and 250 mg POPOP/l toluene) was added and the radioactivity determined in a Nuclear Chicago Mark I scintillation spectrometer. Counting efficiency was determined using the channels ratio technique and varied from 50-80 percent for the solubilized biological samples. All counts were converted to disintegrations per minute.

TLC was performed on precoated silica gel GF plates (5 X 20 cm, 250 μm thick; Analtech) that were activated at 110° C for 60 min before use. Plates were developed in hexane (solvent A) or benzene:ethyl acetate, 12:1 (solvent B) and were sequentially scraped (1 cm bands). The radioactivity present in each segment was determined by counting the silica gel in vials containing 10 ml Fluoralloy (Beckman)-dioxane liquid scintillation cocktail. Radioactive recovery from the plates was normally greater than 95 percent with this procedure. Statistical analysis of the data was performed using Dunnetts' multiple comparisons test. Log transformation was used to equal the variances.

Microsomes were prepared from lobster hepatopancreas or dogfish liver homogenates (in 1.15% KCl buffered to pH 7.6 with 0.013 M HEPES) by centrifugation; microsomal protein concentrations were determined and mixed-function oxidase activities were measured toward aniline, benzphetamine, benzo[a]pyrene, and 7-ethoxycoumarin as described in detail elsewhere (ref. 8).

PCB residue analyses were performed according to Armour and Burke (ref. 9) following caustic hydrolysis of a preliminary extract which removed DDT, PCB's, and related chemicals from the tissues to be studied (ref. 10).

In estimating the degree of metabolism of ^{14}C-2,4,5,2',5'-PCB, 25 percent w/v aqueous homogenates of lobsters hepatopancreas and green gland were extracted four times with equal volumes of warm hexane and/or acidified toluene. Homogenates of livers from dogfish were extracted in a similar manner with benzene.

RESULTS

Of the various lobster tissues investigated, the female egg mass was found to contain the highest residual level of PCB's (table 1) although the hepatopancreas also contained a significant amount. This is interesting since the tomale (i.e., hepatopancrees of the cooked lobster) is considered a delicacy by some people. The PCB concentration in lobster muscle was about an order of magnitude lower than that of egg mass or hepatopancreas and was well below the 5 ppm limitation on PCB residues in human foodstuffs.

Dogfish shark liver also had appreciable PCB residues (table 1), whereas muscle contained only about one-fifth the hepatic concentration (per gram wet weight). These residual PCB's are not unexpected in either dogfish shark liver or lobster hepatopancrees since both tissues have high lipid contents (50 to 60 percent wet weight)(ref. 11).

The specific activity of several lobster tissues, plasma, and feces with time, after parenteral administration of ^{14}C-2,4,5,2',5'-PCB, is shown in table 2, and the percentage of dosed radioactivity present in the various organs at each time point is outlined in table 3. The hepatopancreas contained much higher amounts of radioactivity (per mg tissue) than any other organ (table 2) and accounted for between 91 and 96 percent of the total recovered radioactivity at all times. The half-life ($t_{½}$) for radioactivity in tissues was estimated from semilogarithmic plots of the specific activity (dpm/mg) versus time (days). The tissues could be classified into two categories (table 2): those in which labeled compound had a $t_{½}$ of 3 weeks or longer and those in which the $t_{½}$ was 1 week or less.

Almost all of the radioactivity in lobster hepatopancreas at 2, 4, or 8 weeks after treatment was unchanged 2,4,5,2',5'-PCB. More than 90 percent of this hepatopancreas activity was extracted into hexane after shaking homogenates four times with this solvent (93 ± 3%, mean ± SD, n = 4). Over 95 percent of the hexane soluble radioactivity cochromatographed with authentic 2,4,5,2',5'-PCB (Analabs) on TLC plates developed in solvent A (R_f 0.7-0.8) or solvent B (R_f 0.9-1.0). Similarly, more than 90 percent of the radioactivity in hexane extracts of green glands from lobsters sacrificed 2 or 4 weeks after dosing was 2,4,5,2',5'-PCB, although in this case only about 50 percent of the total activity was hexane and toluene (acidified) extractable, the remainder residing in the aqueous phase of green gland homogenate. Only about 10 percent of the fecal radioactivity was extracted into hexane and acidified toluene (four

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Table 1. Residue levels of polychlorinated biphenyls (PCB's) in some tissues of the lobster *(Homarus americanus)* and the dogfish shark *(Squalus acanthias)*

Species	ppm wet weight PCB's[a]		
Lobster[b]	Egg mass	Hepatopancreas	Muscle
	4.41 (2.78-5.98)	1.57 (1.13-2.27)	0.097 (0.07-0.13)
Dogfish shark[c]	Liver	Muscle	Kidney
	1.87	0.42	0.2[d]

[a]Reported in terms of total Aroclor 1242 and 1254 residues.

[b]Mean and ranges for 4 animals.

[c]Mean values for 2 dogfish weighing 4-6 kg.

[d]Detection limit in dogfish kidney.

Table 2. Specific activity of various lobster tissues and fluids with the following pericardial injection of ^{14}C-2, 4, 5, 2^{1}, 5^{1}, -penta-chlorobiphenyl (0.2 mg/kg)[a]

Tissue	Time after administration					$t_{\frac{1}{2}}$ (days)
	24 hr	1 wk	2 wk	4 wk	8 wk	
	dmp/mg tissue or /ul plasma					
Hepatopancreas	183.4 ± 24.7 (6)[b]	192.9 ± 33.4 (7)	170.9 ± 40.0 (5)	131.2 ± 34.7 (6)	77.4 ± 28.4 (9)	45
Green gland	20.1 ± 13.0 (6)	8.4 ± 2.4 (7)	6.1 ± 1.9 (5)	4.8 ± 1.2 (6)	3.4 ± 1.4 (9)	<7
Intestine	14.0 ± 7.0 (6)	14.3 ± 5.9 (7)	17.6 ± 18.7 (5)	4.3 ± 0.9 (6)	2.2 ± 1.5 (9)	23
Heart	8.8 ± 5.4 (6)	2.7 ± 0.4 (7)	2.2 ± 0.7 (5)	1.6 ± 0.3 (6)	1.1 ± 0.3 (9)	<7
Tail muscle	5.1 ± 1.8 (6)	1.6 ± 0.1 (7)	1.3 ± 0.3 (5)	0.8 ± 0.2 (6)	0.4 ± 0.2 (9)	<7
Claw muscle	5.0 ± 3.1 (6)	1.5 ± 0.2 (7)	1.3 ± 0.1 (5)	0.8 ± 0.1 (5)	0.6 ± 0.2 (9)	<7
Stomach	4.5 ± 1.1 (6)	4.8 ± 2.1 (7)	4.3 ± 2.0 (5)	2.2 ± 1.0 (6)	1.3 ± 0.4 (9)	28
Gill	3.4 ± 1.8 (6)	3.2 ± 1.5 (7)	3.1 ± 2.3 (5)	1.4 ± 0.9 (6)	0.8 ± 0.3 (9)	24
Male gonad	8.3 (1)	1.6 (1)	2.9 (2)	2.1 (2)	1.1 ± 0.2 (5)	<7
Egg masses	16.6 ± 9.7 (5)	7.7 ± 6.6 (6)	10.2 ± 6.0 (3)	8.6 ± 4.0 (6)	7.7 ± 4.0 (4)	39
Plasma	1.33 ± 0.41 (6)	0.49 ± 0.17 (7)	0.62 ± 0.07	0.36 ± 0.13 (5)	0.20 ± 0.05 (9)	<7
Feces	--	--	142.5 ± 70.7 (5)	21.5 ± 6.6 (6)	20.1 ± 10.5 (9)	

[a]Data from Bend et al. (ref. 12).

[b]Mean ± SD (N).

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Table 3. Percent administered radioactivity remaining in tissues of lobster with time after pericardial injection of ^{14}C-2, 4, 5, 2', 5'-pentachlorobiphenyl (0.2 mg/kg)[a]

Tissue	Time after injection				
	24 hr	1 wk	2 wk	4 wk	8 wk
Hepatopancreas	61.4 ± 8.3 (6)[b]	60.5 ± 10.8 (7)	61.4 ± 14.6 (5)	55.2 ± 13.4 (6)	31.2 ± 12.5 (9)
Green gland	0.19 ± 0.12 (6)	0.07 ± 0.01 (7)	0.07 ± 0.03 (5)	0.05 ± 0.01 (6)	0.04 ± 0.02 (9)
Intestine	0.10 ± 0.09 (6)	0.11 ± 0.05 (7)	0.21 ± 0.27 (5)	0.04 ± 0.01 (6)	0.03 ± 0.02 (9)
Heart	0.09 ± 0.04 (6)	0.03 ± 0.01 (7)	0.04 ± 0.02 (5)	0.02 ± 0.01 (6)	0.01 ± 0.01 (9)
Tail muscle	4.36 ± 0.84 (6)	1.57 ± 0.26 (7)	1.54 ± 0.41 (5)	0.96 ± 0.26 (6)	0.45 ± 0.02 (9)
Stomach	0.36 ± 0.14 (6)	0.53 ± 0.39 (7)	0.45 ± 0.22 (5)	0.20 ± 0.10 (6)	0.12 ± 0.04 (9)
Gill	0.61 ± 0.31 (6)	0.59 ± 0.31 (7)	0.60 ± 0.41 (5)	0.25 ± 0.15 (6)	0.16 ± 0.07 (9)
Male gonad	0.06 (1)	0.02 (1)	0.03 (2)	0.04 (2)	0.01 ± 0.01 (5)
Egg masses	0.48 ± 0.34 (5)	0.37 ± 0.56 (6)	0.46 ± 0.44 (3)	0.64 ± 0.38 (4)	0.67 ± 0.53 (4)
Mean total recovery	67.6%	64.8%	64.8%	57.4%	32.7%

[a]Data from Bend et al. (ref. 12).

[b]Mean ± SD (N).

extractions with each solvent), but about 70 percent of the organic extractable material was chromatographically identical to 2,4,5,2',5'-PCB. Neither the water soluble fecal metabolites nor green gland metabolites were investigated further.

Total recovery experiments in which lobster carcasses were homogenized after dissection accounted for as much as 90 percent of the injected radioactivity.

The specific radioactivity (dpm/mg tissue) was higher in dogfish shark liver than in any other tissue following parenteral administration of ^{14}C-2,4,5,2',5'-PCB (table 4). There was an apparent increase in specific radioactivity of liver between 6 and 24 hours, concomitant with a statistically significant decrease in the radioactivity of most other tissues. Liver radioactivity remained at, or above, the 6-hour level throughout the course of the experiment (20.3 dpm/mp at 12 days, the longest period sharks could be maintained).

At all times subsequent to administration of ^{14}C-2,4,5,2',5'-PCB to dogfish sharks, almost all of the recovered radioactivity was located in the liver (table 5), and initially all of the benzene extractable activity from hepatic tissue was unchanged ^{14}C-2,4,5,2',5'-PCB. Only trace amounts of radioactivity were present in all other tissues (less than 1 percent) with the exception of muscle which contained 7 to 9 percent of the dosed activity 3 and 7 days after treatment. This observation was attributable more to the large muscle content of the dogfish (about 23 percent) (ref. 14) than to the specific radioactivity of the tissue.

Urine was collected continuously from a few sharks with cannulated urinary papillae. Only trace amounts of radioactivity were excreted in the urine of these fish after injection of ^{14}C-2,4,2',5'-PCB. The delayed biliary excretion of radioactivity and the excretion of only very small quantities of biliary metabolites suggested that dogfish sharks biotransformed the dosed compound very slowly. Unaltered 2,4,5,2',5'-PCB was not excreted in the bile and the biliary radioactivity was not converted to organic soluble material by β-glucuronidase hydrolysis indicating that glucuronide conjugates of phenols were not major biliary metabolites.

The decline in blood radioactivity following the intravenous administration of ^{14}C-2,4,5,2',5'-PCB is illustrated in figure 1. The disappearance of radioactivity follows a multiple exponential decay relationship with time. Using least squares technics (fig. 2), we resolved this blood decay curve into three components by computer analysis. The $t_{½}$ of ^{14}C-2,4,5,2',5'-PCB was estimated for each component; the α, β, and γ components of the radioactivity disappearance curve had $t_{½}$ values of 4, 27, and 730 minutes, respectively. It is interesting that the α and β components for blood disappearance of the PCB isomer tested had similar $t_{½}$ values to those reported by Dvorchik and Maren (ref. 14) for DDT blood disappearance in this same species (3 and 30 minutes, respectively).

The initial, rapid phase of radioactivity removal from blood after injection of ^{14}C-2,4,5,2',5'-PCB is probably related to the rapid uptake into liver.

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Table 4. Specific activity of various dogfish shark tissues and fluids with time following intravenous injection of ^{14}C-2, 4, 5, 2^{1}, 5^{1} -pentachlorobiphenyl (0.03 mg/kg)[a]

Tissue	Time after administration			
	6 hr[b]	24 hr[b]	3 days[b]	7 days[c]
	dpm/mg tissue or /µl fluid			
Liver	14.00 ± 2.15	21.63 ± 4.80	18.98 ± 5.77	14.05 ± 2.39
Kidney	5.39 ± 0.72	1.04 ± 0.22[e]	0.65 ± 0.05[e]	0.49 ± 0.05[e]
Salt gland	3.12 ± 0.91	0.72 ± 0.07[e]	0.60 ± 0.07[e]	0.36 ± 0.05[e]
Pancreas	2.31 ± 0.77	0.49 ± 0.08[e]	0.53 ± 0.16[e]	0.33 ± 0.05[e]
Spleen	1.86 ± 1.04	0.59 ± 0.05[e]	0.61 ± 0.08[e]	0.46 ± 0.06[e]
Gill	1.84 ± 0.38	0.78 ± 0.05[e]	0.55 ± 0.13[e]	0.70 ± 0.21[d]
Brain	0.92 ± 0.30	1.07 ± 0.24[e]	0.40 ± 0.16[e]	0.16 ± 0.03[e]
Heart	0.82 ± 0.10	0.36 ± 0.02[e]	0.41 ± 0.11[e]	0.15 ± 0.04[e]
Muscle	---	---	0.87 ± 0.42	0.66 ± 0.08
Blood	0.80 ± 0.02	0.32 ± 0.08[d]	0.50 ± 0.23	0.14 ± 0.13[c]
Bile	0.41 ± 0.21	1.35 ± 0.51[d]	2.71 ± 0.58[e]	12.03 ± 4.83[e]
CSF	0.02 ± 0.03	0.03 ± 0.05	0.06 ± 0.05	0.04 ± 0.04

[a]Data from Hart et al. (ref. 13).

[b]Mean ± SD (N = 3).

[c]Mean ± SD (N = 4).

[d]$p < 0.05$ compared with 6 hr.

[e]$p < 0.01$

Table 5. Percent administered radioactivity remaining in tissues of Dogfish shark with time after intravenous injection of ^{14}C-2, 4, 5, 2^{1}, 5^{1} -pentachlorobiphenyl (0.03 mg/kg)[a]

Tissue	Time after injection			
	6 hr[b]	24 hr[b]	3 days[b]	7 days[c]
Liver	75.22 ± 6.11	94.02 ± 24.65	79.55 ± 12.77	89.42 ± 11.17
Kidney	0.43 ± 0.16	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.02
Salt gland	0.08 ± 0.02	0.02 ± 0.002	0.01 ± 0.001	0.01 ± 0.001
Pancreas	0.25 ± 0.03	0.05 ± 0.02	0.05 ± 0.02	0.03 ± 0.004
Spleen	0.21 ± 0.04	0.08 ± 0.02	0.06 ± 0.02	0.07 ± 0.03
Brain	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.001	0.01 ± 0.002
Heart	0.05 ± 0.01	0.01 ± 0.002	0.02 ± 0.004	0.01 ± 0.003
Mean total recovery	76.3%	94.3%	79.8%	89.6%

[a]Data from Hart et al. (ref. 13).

[b]Mean ± SD (N = 3).

[c]Mean ± SD (N = 4).

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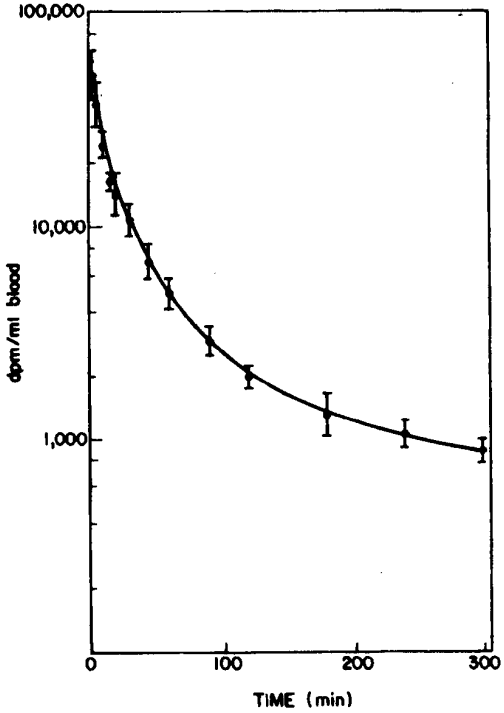


Figure 1. Decline in blood activity following the intravenous administration of ^{14}C-2, 4, 5, 2', 5'-PCB.

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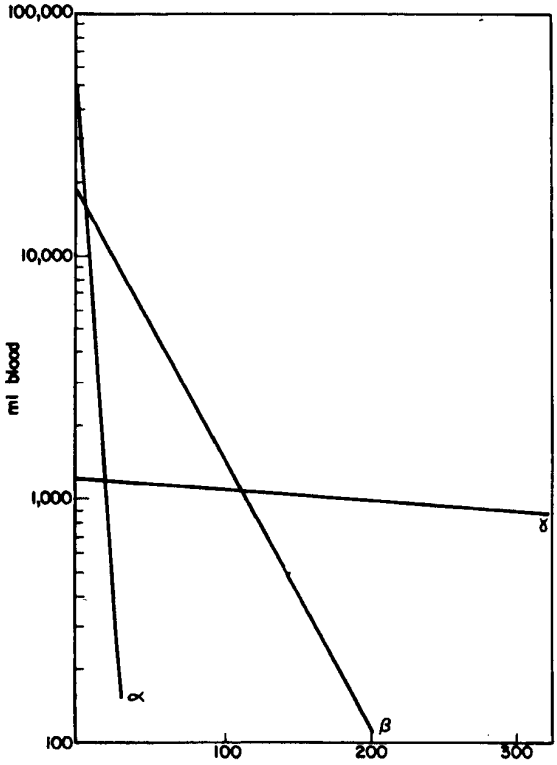


Figure 2. Three components of blood decay curve.

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Microsomes prepared from lobster hepatopancreas had no detectable aniline hydroxylase, benzphetamine N-demethylase, 7-ethoxycoumarin O-deethylase, or benzpyrene hydroxylase activity (table 8), whereas dogfish shark liver microsomes had appreciable (but low, relative to the little skate, *Raja erinacea*, another marine elasmobranch indigenous to Maine) mixed-function oxidase activity toward each substrate tested. These assays were run at 30° C, the optimal in vitro temperature for 7-ethoxycoumerin O-deethylation in the dogfish (ref. 8). At 12° C, which is within the range of summer water temperature in Maine, specific 7-ethoxycoumarin O-deethylase activity was only about 20 percent of that found at 30° C.

DISCUSSION

Although the accumulation and toxicity of commercial PCB mixtures, consisting of many isomers of varying chlorine content, were demonstrated in many aquatic species (refs. 15-22) few such studies have utilized single, purified PCB isomers. Hutzinger et al. (ref. 23) demonstrated that 4-chlorobiphenyl, 4,4'-dichlorobiphenyl, and 2,2',5,5'-tetrachlorobiphenyl were hydroxylated by rat and pigeon but not by brook trout, a freshwater species. No hydroxylated metabolites of 2,2',4,4',5,5'-hexachlorobiphenyl were found in rat, pigeon, or brook trout.

In previous studies from our laboratory, we found that many marine species have some hepatic microsomal mixed-function oxidase activity, but that this activity is generally considerably lower than that found in mammalian liver (refs. 8, 24-26). Reduced rates of oxidative xenobiotic metabolism in marine vertebrates and invertebrates would partially account for the persistence of lipophilic compounds, such as the PCB's, in these species.

The distribution, excretion, and metabolism of ^{14}C-2,4,5,2',5'-PCB in the rat was studied by Matthews et al. (refs. 27-31), working in our laboratory. This compound is metabolized readily by rats and the major metabolites are 3-hydroxy-, 3',4'-dihydroxy-, and 3',4'-dihydro-3',4'-dihydroxy-2,4,5,2',5'-pentachlorobiphenyls; the first half-life in rats is only about 2.5 days. This varies markedly from both the lobster (tables 2 and 3) and the dogfish shark (tables 4 and 5), where excretion of radioactivity occurs very slowly and the fatty hepatopancreas and liver, respectively, act as storage depots for ^{14}C-2,4,5,2',5'-PCB.

The very slow metabolism (and excretion) of ^{14}C-2,4,5,2',5'-PCB in the lobster is probably related to the very low microsomal mixed-function oxidase activities of lobster hepatopancreas (table 8). In separate experiments, we found only traces of benzpyrene hydroxylase or aniline hydroxylase activity in gill or hepatopancreas homogenate. The much lower concentrations of PCB's (tables 1 and 2) in lobster muscle and dogfish muscle (vs. hepatopancreas or egg mass) suggest that portions of marine species might be used for foodstuffs even in certain organs exceed the FDA recommended

Table 8. Mixed-function oxidase activity in lobster hepatopancreas and dogfish shark liver microsomes[a]

Species	Aniline Hydroxylase[b]	Benzphetamine N-demethylase[b]	7-Ethoxycoumarin O-deethylase[b]	Benzpyrene Hydroxylase
Lobster, *Homarus americanus*	0.01 (3)[d,e]	0.06 (3)	0.01 (3)	0.01 (3)
Dogfish Shark, *Squalus acanthias*	0.07 ± 0.01 (3)[f]	0.15 ± 0.05 (3)	0.08 ± 0.02 (3)	0.07 ± 0.02 (3)

[a] Data taken from Pohl et al. (ref. 8).

[b] Nmoles/min/mg microsomal protein.

[c] Fluorescence units/min/mg microsomal protein.

[d] Minimum metabolism detected by assay procedure used.

[e] Microsomes from three lobsters were tested separately.

[f] Mean ± SD (N).

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PCB levels, provided that care is taken in preparation and that no PCB metabolites or degradation products with high toxicity to humans are present (ref. 32).

Although hepatic microsomes from dogfish shark have mixed-function oxidase activity, this activity is low relative to mammals and some other marine species (ref. 8). Furthermore, dogfish liver is very fatty and the microsomal protein yield is very low (only 1-3 mg protein/g liver vs. 6-15 mg/g liver in the little skate), meaning that whole liver xenobiotic-metabolizing activity per gram tissue in the dogfish is even lower relative to the skate than is specific activity (per mg microsomal protein).

The liver appears to function both as the major site of ^{14}C-2,4,5,2',5'-PCB uptake and storage in the dogfish shark, probably due to its high lipid content. The delayed excretion may be related to poor partitioning of the compound from liver lipid into metabolically active parenchymal cells, and to the low mixed-function oxidase activity, especially at 12° to 15° C which is the Maine water temperature in summer.

The persistence of trace doses of ^{14}C-2,4,5,2',5'-PCB, an isomer that is metabolized rapidly by rats, in the two marine species studied suggests that the problem of PCB residues in marine and freshwater species will be with us for some time, especially in the face of continued PCB release into the aquatic environment.

ACKNOWLEDGMENT

We are grateful to Ms. S. Bend, Ms. T. Devereux, and Ms. R. Pohl for excellent technical assistance and to Dr. J. Haseman for the statistical analysis.

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