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Effect of Polychlorinated Biphenyls (PCB's) on the Eggshells of Ring Doves

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Since their discovery in the ecosystem by Jensen in 1966(1) the polychlorinated biphenyls (PCB's) have been found to be widely distributed, often in considerable amounts. These data have been recently reviewed(2). Since the formulae of PCB's are similar to that of DDT it has been suggested that their actions may be similar. The outstanding effect of DDT and its metabolites on avian reproduction is to cause the thinning of egg-shells. This phenomenon has been recently reviewed by Ratcliffe(3), and it physiological basis discussed by Peakall(4).

One group of Ring Doves (Streptopelia risoria) were fed a diet containing 10 ppm PCB's (Arochlor 1254, kindly supplied by the Monsanto Chemical Company) for six months and the ashed (16 hrs, 1000 C) egg-shell weights compared to those from a group of control birds. No consistent changes in egg-shell weights were noted over this period, nor did the first and second egg of the clutch differ significantly. The conditions for maintaining birds has been given previously(5). In order to overcome the effect of individual variation, a second experiment was performed in which eggs were collected from the individual birds under study both before and after being put on a diet containing 10 ppm PCB's. In the third experiment 25mg PCB's (equivalent to 160mg/kg) were injected intraperitoneally one to four days before egg-laying and ashed egg-shell weights compared to those of eggs laid by the same individual before injection. Previous experiments had shown that injection of the carrier alone was without effect. The results are summarised in table 1 and it is clear no effect was observed.

TABLE 1

	Ashed shell weight in mg	
	Experimental	Control
Diet 10 ppm PCB's Separate controls	229.8 ± 4.5 (24)	231.8 ± 2.8 (26)
Diet 10 ppm PCB's Individuals acting as own controls	230.2 ± 3.2 (14)	232.0 ± 3.9 (14)
Injection experiment 25mg ip PCB's	224.1 ± 8.6 (9)	228.3 ± 3.7 (10)

Figures are means, standard error, and sample size.

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In the case of the doves injected with PCB's, the carbonic anhydrase levels in the oviduct were determined by a method previously described(5). No difference from control values was observed. Residue analyses were carried out on egg contents and at the end of the experiment brains were taken for analysis. The average residue level in the eggs from the first experiment in which the birds were on diet for approximately 35 days was 4.81 ppm with a standard error of 1.08. Fuller details of this work will be reported elsewhere.

Although experimental studies have not previously been reported some attempt has been made to determine the effect of PCB's on egg-shell thinning by regression analysis from field samples(6,7). These studies are complicated by the presence of considerable amounts of DDE and lesser amounts of other pesticides. Anderson and co-workers(6) concluded that DDE has a greater effect on shell thickness than PCB's based in their studies on Double-crested Cormorants (Phalacrocorax auritus) and White Pelicans (Pelecanus erythrorhynchos) in western Canada. Vermeer and Reynolds(7) found no significant correlation between shell thickness and PCB's residues for the Great Blue Heron (Ardea herodias), but their range of concentrations of PCB's were too small for this regression analysis to be convincing. Risebrough (private communication) has come to the same conclusion based on his data for the Brown Pelican (Pelecanus occidentalis) in California. In this case the range of PCB's concentrations was large.

It is concluded that PCB's do not play a significant role in the phenomena of egg-shell thinning.

Acknowledgements

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Metabolism of a Polychlorinated Biphenyl (Aroclor® 1254) Mixture in the Rat

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Polychlorinated biphenyls (PCB's) have been used in industry since at least 1930. In 1955, von Oettingen (1) reviewed the literature on the toxicity of these compounds. Industrial workers have developed lesions of chloracne as a result of leaking vapours of a chlorinated biphenyl (Aroclor(R)*) used as a heat-exchange material (2).

Today the problem is not restricted to exposure of industrial workers as polychlorinated biphenyls are widely dispersed in fish and wild life (3-11). Westoo et al. (11) found low levels of PCB (90%, <0.1 mg per kg fat) in a large number of foodstuffs. They also reported that all 22 samples of human milk analyzed contained PCB residues with 11 samples between 0-0.5 and the other 11 between 0.6-1.0 mg per kg fat. Therefore, there is a need to study the metabolism, distribution, storage, effect on reproduction, etc., of PCB's in mammals. This paper will report on the metabolism and distribution of Aroclor 1254 in normal and carbon tetrachloride-treated rats.

Experimental

Experiment I

The distribution of PCB's in various tissues of the rat and the effect of liver damage on their metabolism, was studied in twenty male Wistar rats randomized into four groups and treated as described in TABLE I.

Experiment II

The effect of Aroclor 1254 on the organ weights, fat content of the liver and on the potentiation of the toxicity of carbon tetrachloride was investigated in thirty eight Wistar rats divided into four groups and treated as described in TABLE II.

In Experiments I and II, the rats were housed in air conditioned rooms and were supplied with food and water *ad libitum*. The rats were killed by decapitation, blood collected, and brain, liver, heart, spleen, kidneys, testes and omental fat removed, weighed and frozen pending analyses.

*Aroclor(R), Monsanto Company (U.S.A.), registered trade name for polychlorinated biphenyls.

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TABLE I

Dosing regimen for experiment I

Group[a]	1	2	3	4
Day 1	Oil[b]	Oil[b]	CCl_4[c]	CCl_4[c]
Day 2	PCB[d]	PCB[d]	PCB[d]	PCB[d]
Day 3	Oil[e]	Oil	CCl_4[f]	CCl_4[f]
Day 4	Killed	--	Killed	--
Day 8	--	Oil[e]	--	CCl_4[f]
Day 24	--	Killed	--	Killed

[a] Each group contained five male rats, Wistar strain, av. b.w. 375 g.
[b] Corn oil, 2 ml per kg, administered orally.
[c] A 1+1 CCl_4:corn oil solution, 2 ml per kg, administered orally.
[d] A 500 mg per ml corn oil solution of Aroclor 1254, administered orally at 500 mg per kg.
[e] Corn oil, 1 ml per kg, administered orally.
[f] A 1+1 CCl_4:corn oil solution, 1 ml per kg, administered orally.

TABLE II

Dosing regimen for experiment II

Group[a] 1	Nil
Group[b] 2	PCB[c] on day 2
Group[d] 3	CCl_4[e] on days 1, 3, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 47
Group[d] 4	CCl_4 on days 1, 3, and 8 and PCB[c] on day 2

[a] Five male rats, Wistar strain, av. b.w. 410 g, killed on day 1.
[b] Fifteen male rats, Wistar strain, av. b.w. 440 g, 5 killed on days 8, 12, and 47.
[c] A 500 mg per ml corn oil solution of Aroclor 1254 administered orally at 500 mg per kg.
[d] Nine male rats, Wistar strain, av. b.w. 442 g.
[e] A 1+1 CCl_4:corn oil solution, 2 ml per kg administered orally on day 1 and 1 ml per kg on other days.

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Polychlorinated biphenyl analyses. Tissues were blended with 100 ml hexane and 50 g anhydrous sodium sulfate for 10 minutes. The extracts were filtered, concentrated to 10 ml, 3 ml of conc. H_2SO_4 - fuming H_2SO_4 (1:1) added and shaken. The hexane solutions were dried and neutralized with 4 g of anhydrous sodium sulfate-sodium carbonate (10:1). The solutions were subjected to GLC-EC analyses on a Varian Aerograph Model 600D gas chromatograph, fitted with a coiled 4' x 1/4" O.D. glass column containing Chromosorb W, 80-100 mesh, coated with 4% SE-30 and 6% QF-1. The nitrogen flow rate was 120 ml per minute with column and injection temperatures of 193 and 225°C, respectively. Aroclor 1254 standards and test solutions were chromatographed and the concentration of PCB's in the test solutions determined by comparing the heights of the six major peaks of the standards with corresponding peak heights of the test solutions.

Problems were not encountered with sensitivity during analyses and the lower limit of detection would depend on the tissue being analyzed. Hexane and acetone gave equal recoveries of Aroclor residues. Quadruplicate analyses of a rabbit liver gave values of 431, 438, 464, and 474 ppm. Recoveries of spiked samples were greater than 95%.

Lipid was determined by the method of Bligh and Dyer (12).

Results and Discussion

Experiment I

From day 1 to day 4 the rats dosed with corn oil and Aroclor 1254 (groups 1 and 2) or carbon tetrachloride and Aroclor 1254 (groups 3 and 4) lost an average of 19 and 55 g (b. w.), respectively. On day 8, group 2 rats had regained their lost weight, while group 4 rats continued to lose weight. Four of the five rats in group 4 which received the oral dose of carbon tetrachloride on day 8 died within the next 24 hours. However, by day 28 the 1 remaining rat in group 4 and the five rats in group 2 had gained 20 and an average of 30 g, respectively. The livers of the group 2 rats were enlarged and averaged 5.32±0.30% of their body weight.

Residues of Aroclor 1254 (expressed as ppm wet tissue, TABLE III) were found in all tissues analyzed, with fat and blood having the greatest and least concentration, respectively.

The residues in the blood, testes, liver, kidney and heart were significantly greater in the group 3 rats than in the group 1 rats. This shows that the liver is the main site of Aroclor 1254 metabolism and rats with carbon tetrachloride damaged livers are not able to metabolize this mixture as rapidly as rats with normal livers. The residues in the spleen, brain and fat were similar for both groups. The residues in the brain, liver,

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spleen, blood, testes, heart, kidney and fat of group 2 rats (killed on day 24) were 10, 16, 20, 21, 22, 24, 36 and 67%, respectively, of those of group 1 (killed on day 4). These values show that the PCB residues were being cleared from the various tissues at different rates.

TABLE III

Residues in tissues of rats orally dosed with Aroclor 1254 (500 mg/kg)

	Residue found (ppm, wet tissue)			
Group	1	2	3	4
Blood	1.96[a] ± 0.23[b]	0.42 ± 0.07	3.85 ± 0.46	0.25[c]
Testes	19.22± 0.59	4.30± 0.44	33.18± 1.35	5.62
Heart	24.16± 2.84	5.83± 0.53	62.40± 4.37	6.17
Spleen	29.17± 3.44	5.82± 1.17	36.60± 4.39	--
Kidney	31.14± 2.09	11.20± 1.76	57.38± 3.91	11.08
Brain	39.98± 5.91	4.01± 0.31	41.91± 3.30	5.96
Liver	115.66± 10.55	18.85± 1.65	796.47± 64.96	18.79
Liver[d]	1868.14±166.63	--	6137.64±556.06	--
Fat	996.16± 98.58	672.66±155.12	900.46±106.16	1149.05

[a]Mean of five values.
[b]Standard error of the mean.
[c]Single value.
[d]Ppm on a fat basis.

GLC-EC tracings from 24 ng of Aroclor 1254 and from the residue found in the liver of a group 2 rat, are shown in Figure 1. The amount (%) that each of the six major peaks contribute to the total residue in the tissues is presented in TABLE IV.

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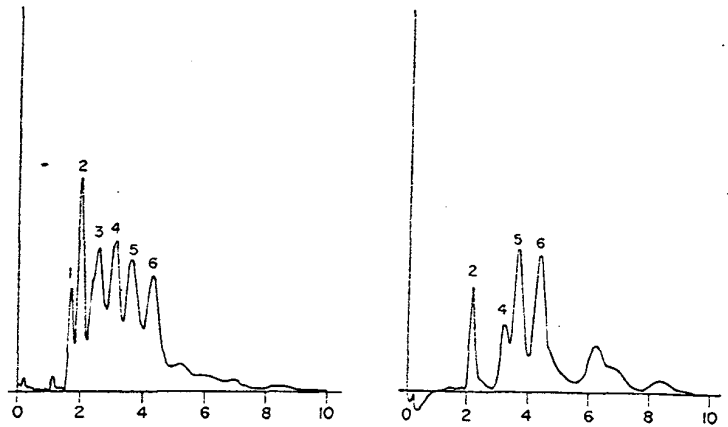

Figure 1 GLC-EC tracings from 24 ng of Aroclor 1254 (left) and from the residue found in the liver of a group 2 rat.

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TABLE IV

The amount (%) that each of the six major peaks of the Aroclor 1254 standard contribute to the total residue in the tissues

Peak number	Percent 1	2	3	4	5	6	Percent 1	2	3	4	5	6
Aroclor 1254	12	25	18	17	15	13	12	25	18	17	15	13
			Group 1						Group 2			
Spleen	6	21	9	22	21	21	3	17	2	11	34	32
Testes	3	15	6	21	27	28	1	19	2	11	35	32
Liver	2	11	3	30	28	27	0	25	2	18	26	28
Kidney	8	21	11	21	19	19	0	18	2	14	34	32
Fat	7	25	11	25	17	16	1	19	4	20	28	28
Brain	1	13	5	23	30	29	2	19	4	12	34	29
Blood	4	13	6	24	26	26	1	20	3	12	33	31
Heart	4	17	6	21	26	26	1	17	2	8	31	29
			Group 3						Group 4			
Spleen	7	25	14	19	18	17	--	--	--	--	--	--
Testes	6	23	14	19	19	19	1	20	4	12	31	32
Liver	4	21	11	21	22	21	0	19	4	20	26	31
Kidney	8	22	15	18	18	17	1	21	4	8	32	34
Fat	10	29	16	21	13	12	1	20	5	20	26	28
Brain	6	25	13	20	18	18	1	22	4	11	30	31
Blood	5	22	14	22	18	18	2	22	3	11	29	33
Heart	5	27	15	18	18	17	1	20	3	10	32	34

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Figure 1 and TABLE IV show that the components of the Aroclor 1254 mixture with the shorter retention times, peaks 1, 2 and 3, and presumably with the lowest chlorine contents (10) were metabolized to a greater degree than those with the longer retention times. This observation agrees with that reported in studies with Phenochlor DP6* fed to Japanese Quail (7). In group 2, peak 2 accounted for 25, 20, 19 and 19% of the residue in the liver, blood, brain and testes, respectively, whereas in group 1 the percents were 11, 13, 13 and 15%, respectively. Thus, the metabolism of this component (peak 2) was very slow between day 4 and 24 or one or more of the other components of the mixture were metabolized to the peak 2 component. The residue pattern in the fat of animals treated with carbon tetrachloride (Group 3) was similar to that of the standard Aroclor 1254 indicating little metabolism.

Expression of the Aroclor 1254 residues in the tissues relative to those in blood (TABLE V) shows that the ratios depend on the length of time following the oral dose (kidney, brain, liver and fat, group 1 vs 2) and the metabolic activity of the liver (liver and fat, group 1 vs 3).

TABLE V

Concentrations of the Aroclor 1254 residues in tissues relative to those in blood

Group	1	2	3	4
Blood	1.00	1.00	1.00	1.00
Testes	9.81	10.24	8.62	22.48
Heart	12.33	13.88	16.21	24.68
Spleen	14.80	13.86	9.51	--
Kidney	15.89	26.67	14.90	44.32
Brain	20.40	,9.55	10.89	23.84
Liver	59.17	44.88	206.88	75.16
Fat	508.24	1601.57	233.89	4596.20

*Phenochlor DP6, Prodelec's (France) registered trade name for polychlorinated biphenyls.

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However, since blood samples are relatively easy to obtain they could be used for estimating the residue in other tissues, providing the investigator is aware of the limitations of the estimates.

Experiment II

In Experiment I no rats received carbon tetrachloride alone, therefore experiment II was conducted to determine whether Aroclor 1254 (a) potentiates the toxicity of carbon tetrachloride and (b) alters the weights of other organs besides the liver.

Eight of the 9 rats in group 4 died within 24 hours of receiving the carbon tetrachloride on day 8, while none of the rats in group 3 died up to day 47, when they were killed. Thus, it is concluded that Aroclor 1254 potentiated the toxicity of carbon tetrachloride in a manner similar to that reported for phenobarbital (13-15) and for DDT (13, 15).

Treatment with Aroclor 1254 evoked a number of significant changes in organ weights (TABLE VI), the most consistent being an increase in liver weight.

TABLE VI

Significant differences (Student Fisher's t test) found in organs weights from rats treated with Aroclor 1254 and untreated rats

Day killed	8	12	47
Organ	Probability		
Spleen	nil	nil	$<0.01^{a}$
Liver	$<0.005^{a,b}$	$<0.005^{a,b}$	$<0.005^{a,b}$
Heart	nil	$<0.05^{b}$	$<0.05^{b}$
Kidney	-	$<0.05^{a}$	-
Testes	-	$<0.05^{a}$	-

[a] Expressed as % body weight.
[b] Expressed as actual organ weight.

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This agrees with results from experiments in this laboratory on rabbits treated with PCB's, which will be reported elsewhere. More differences were observed on day 12 than on days 8 and 47. The only organ to significantly decrease in weight was the spleen on day 47.

The results of the lipid analyses of some livers from experiments I and II are presented in TABLE VII.

TABLE VII

Percent lipid of rat livers from experiments I and II

Treatment	Lipid (%)
Nil, killed on day 1[a]	4.45[b]±0.008[c]
Aroclor 1254 on day 2, killed on day 4[d]	6.19 ±0.23
Carbon tetrachloride on day 1, killed on day 4[e]	13.22 ±1.17
Aroclor 1254 on day 2, killed on day 8[f]	8.35 ±0.99
Aroclor 1254 on day 2, killed on day 12[f]	7.78 ± 0.38

[a] Experiment II, group 1.
[b] Mean of five values.
[c] Standard error of the mean.
[d] Experiment I, group 1.
[e] Experiment I, group 3.
[f] Experiment II, group 2.

In experiment II, the lipid content of livers from rats killed 6 and 10 days after receiving the Aroclor were significantly higher than the controls ($P<0.005$, student Fisher's t test). Tanaka et al. (16) reported that chlorobiphenyls given orally (0.1 g per kg per day) to rats for four weeks caused loss of body weight, hepatomegaly and marked increase in serum lipid components. As expected, livers from rats which received the carbon tetrachloride and Aroclor 1254 had a very high content of lipid.

A number of the tissues from the rats killed on day 47 were analyzed for PCB residues (TABLE VIII).

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TABLE VIII

Residues in tissues of rats 45 days after being orally administered a single dose of Aroclor 1254 (500 mg/kg)

Residue Group[a]	ppm, wet tissue 2	Relative to Blood 2
Blood	0.18[b] ±0.05[c]	1.00
Heart	2.71 ±0.61	15.06
Kidney	3.39 ±0.29	18.83
Brain	4.19 ±0.34	23.28
Liver	16.04 ±3.79	89.11
Fat	397.30 ±41.86	2207.22

[a]Group 2 of Experiment II killed on day 47.
[b]Mean of five values.
[c]Standard error of the mean.

The residues in the blood, heart, kidney, brain, liver and fat were 9, 11, 11, 10, 14 and 40% respectively, of those found in group 1, Experiment I rats (TABLE I). The tissue-blood residue ratios again show these values depend on the length of time following dosing. The uneven rate of metabolism of the individual Aroclor components was again noted. For example, the amount (%) that each of the six major peaks contributed to the total residue in the fat was 0, 15, 1, 12, 40 and 34, compared to 12, 25, 18, 17, 15, and 13 for Aroclor 1254 standard.

Summary

Male rats were orally dosed with Aroclor 1254 and residues were found in all tissues analyzed, with the greatest concentration in the fat. The GLC-EC pattern of the residues was different from the standard mixture administered, indicating that all components were not metabolized at the same rate. Higher residues were found in the carbon tetrachloride-treated rats. Aroclor 1254 residues in the brain, spleen, blood, testes, heart, kidney and fat were reduced by 90, 84, 80, 79, 78, 76, 64 and 33% respectively in 20 days. Aroclor 1254 significantly increased the size of the liver and also the percent lipid in the liver. Aroclor 1254 was found to potentiate the toxicity of carbon tetrachloride in the rat.

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Acknowledgment

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Chronic Toxicity, Uptake, and Retention of Aroclor® 1254 in Two Estuarine Fishes[1]

by D. J. Hansen, P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr., and P. D. Wilson

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Evidence of the global ubiquity of polychlorinated biphenyls (PCB's) continues to mount. Since the first concern about these persistent industrial pollutants was voiced (1), PCB's have been found in fish-eating birds and their eggs in England (2), in seals and porpoises in Scotland (3), in fish-eating birds, their eggs, and fish in California (4), and in mussels, fish, and fish eating birds in the Netherlands (5). Workers at this laboratory found a PCB, Aroclor 1254, in the water, sediment, and biota of a local estuary, and reported its acute toxicity to some estuarine organisms (6). Because high residues of this PCB were found in five fishes in the estuary and it was not acutely toxic to juvenile pinfish (Lagodon rhomboides) in laboratory tests, we began chronic toxicity tests to determine effects of low levels of Aroclor 1254 on pinfish and spot (Leiostomus xanthurus), another estuarine fish. Concurrently, we measured uptake and retention of this PCB in six tissues of spot.

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METHODS AND PROCEDURES

Juvenile pinfish and spot were exposed to Aroclor 1254 in a modification of flowing seawater bioassays routinely used in this laboratory (7). Fish were acclimated to test conditions prior to exposure. Each test was repeated at least once, and for each test, an equal number of fish were placed in two aquaria, one control and one experimental. Aroclor 1254 was dissolved in acetone or polyethylene glycol (molecular weight 200) and metered into the experimental aquaria. The same amount of solvent was added to control aquaria. Concentrations of this PCB in water samples taken periodically from experimental aquaria were within $\pm$ 20% of the desired concentration. All fish were regularly fed commercial fish food that contained no detectable PCB residue. (Limit of detectability was < 0.02 parts per million, ppm.) Control fish contained no detectable PCB at the end of each experiment.

The same exposure techniques were used in determining the uptake and retention times of Aroclor 1254 in spot which were exposed for 56 days and then placed in flowing water free of the PCB for 84 days. Ten fish were removed at selected intervals for residue analyses of pooled samples of brain, gills, liver, muscle (all muscle above lateral line on left side of fish), and remaining tissues. Residues in all tissues were summed to compute levels of Aroclor 1254 in the whole fish.

Chemical analyses for residues of Aroclor 1254 in pooled samples of fish tissue were performed in the following manner:
A.) Samples that weighed more than one gram were mixed with sodium sulfate in a blender and extracted for four hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and eluted from a Florisil column (8) with 6% ethyl ether in petroleum ether.
B.) Samples that weighed less than one gram were analyzed by a modification of the Enos (9) micro method. Samples were weighed in glass tissue grinders and extracted with three 2.0 milliliter (ml) portions of acetonitrile. The acetonitrile extracts were combined and diluted with 6.0 ml of distilled water. The mixture was agitated and extracted with three 2.0 ml portions of hexane. The extracts were combined and concentrated to about 0.5 ml. The extract was transferred to a size "B" Chromaflex[2] column (Kontes Glass Co.) containing 1.5 grams of Florisil topped with 1.5 grams of sodium sulfate.

[2] Use of trade names does not constitute endorsement by the U. S. Environmental Protection Agency.

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The extract was eluted from the column with 20.0 ml of 1% ethyl ether in hexane.

All eluates were adjusted to an appropriate volume for analysis by electron capture gas chromatographs equipped with DC-200 columns. The multiple-peaked Aroclor 1254 was quantitated by averaging the heights of five major peaks having a retention time relative to aldrin of 1.31 (peak IV), 1.55 (V), 2.32 (VIII), 2.74 (IX), and 3.27 (X). Interference from DDT and its metabolites was negligible due to the relatively high concentrations of Aroclor 1254 found in the samples. Recovery rates were above 80%. Data in this report were not corrected for percentage recovery.

RESULTS AND DISCUSSION

Mortality

Pinfish and spot died when exposed for 14 - 45 days to 5 parts per billion (ppb) of Aroclor 1254, but spot appeared unaffected by exposure to 1 ppb for periods up to 56 days (Table 1). Onset of death in both species exposed to 5 ppb was delayed. For example, mortality was less than 1% in the first week in 3 of 5 tests, and less than 15% in the other two tests. Further exposure to 5 ppb, however, resulted in mortalities ranging from 41 to 66%. Exposures were terminated after these mortalities occurred and live fish were either placed in water free of Aroclor 1254 or analyzed for this PCB.

Symptoms exhibited by fish prior to death were not those typical of poisoning by organochlorine pesticides (10). Instead, pinfish usually developed fungus-like lesions on the body, especially around the mouth, where hemorrhaging also occurred. Affected spot usually ceased feeding, became emaciated, and developed ragged fins and lesions on the body. Although the presence of one or more of these symptoms was typical, some fish appeared normal until they died. All control fish appeared healthy.

Some fish that survived exposure to 5 ppb of Aroclor 1254 became diseased and died even though they were placed in flowing water free of this PCB. For example, 61% (11 of 18) of the pinfish from a population exposed for 14 days died within two weeks, but there was evidence of healing in survivors. Forty-eight percent (48 of 100) of the spot from a population exposed for 26 days died within one week after being placed in PCB-free water.

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TABLE 1

Test conditions, mortality of fishes, and uptake of Aroclor 1254 in flowing water bioassays

Fish	SL (mm)	Number* Exposed	Concentration	Mortality** 25% Day	Mortality** 50% Day	Mortality** Terminal Percent	Days Exposed	Aroclor 1254 (ppm)*** in fish after exposure	Water Temperature, °C	Salinity, ‰
Spot	25	150	1 ppb	--	--	17	33	17	14 - 16	16 -32
Spot	40	150	1 ppb	--	--	1	56	27	23 - 32	10 -34
Spot	24	50	5 ppb	12	18	51	20	46	11 - 18	16 -32
Spot	24	300	5 ppb	23	26	53	26	120	8 - 10	20 -32
Spot	74	50	5 ppb	28	38	62	45	152	28 - 33	23 -34
Pinfish	30	50	5 ppb	9	12	66	14	14	16 - 22	20 -32
Pinfish	27	54	5 ppb	17	--	41	35	109	22 - 32	14 -34

* An equal number of fish were held in control aquaria. Control mortality never exceeded 7%.

** Mortality of spot exposed to 5 ppb Aroclor was significantly greater in all tests ($p < .001$) than that of control fish (χ^2 values were all greater than 10.83).

*** milligrams per kilogram

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Mortality did not appear directly related to the relative amount (ppm) of Aroclor 1254 stored by fish exposed to 5 ppb, rather the amount of PCB stored increased as duration of exposure increased (Table 1). For example, pinfish exposed for 35 days suffered half the mortality yet stored eight times more chemical than did pinfish exposed for only 14 days. Although mortality of spot (51 - 62%) was similar in three tests of different duration (20 - 45 days), the amount of Aroclor 1254 in the fish (46 - 152 ppm) increased as duration of exposure increased.

Uptake and Retention

Spot exposed to 1 ppb Aroclor 1254 for 56 days rapidly stored this chemical, maximum levels being attained in 14 to 28 days. Thereafter, the relative amount (ppm) was generally constant, whereas the absolute amount (micrograms, µg) continued to increase as the fish grew (Table 2). The liver concentrated the greatest relative amount of this PCB, followed in decreasing order by the gills, whole fish, heart, brain, and muscle. Maximum concentrations in whole spot was 3.7×10^4 times that in the test water. In earlier work with DDT at this laboratory, DDT content in pinfish and Atlantic croaker (Micropogon undulatus) exposed to 0.1 and 1.0 ppb p, p'-DDT in water increased for two weeks, then remained constant at 10^4 to 3.8×10^4 times the exposure concentration (11).

Aroclor 1254 was slowly lost from tissues of spot after they were placed in PCB-free flowing water (Table 2). After 84 days of flushing, the relative amount (ppm) in whole fish dropped 73% and the absolute amount (µg) decreased 61%.

The isomers of Aroclor 1254, with the exception of peak IV, maintained their integrity in spot. The height of peak IV in the standard was 18% of the total height of the five peaks used for quantitation. Regardless of the tissue analyzed, it decreased to 14% of the total in exposed fish and 7% in fish maintained in water free of PCB for retention studies.

CONCLUSIONS

Our results suggest that chronic exposure to Aroclor 1254 increased susceptibility of test pinfish and spot to disease, and also appeared to be toxic to these fish. This PCB is rapidly stored by pinfish and spot, and persists in tissues for approximately three months. Our findings emphasize the need for further study on the effect of chronic exposure of aquatic organisms to polychlorinated biphenyls.

of control fish (% values were all greater than 10.83).

milligrams per kilogram

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TABLE 2

Levels of Aroclor 1254 in ppm, wet weight, in spot exposed to 1 ppb of this chemical (each sample consisted of tissues from 10 fish). Total micrograms of Aroclor in parenthesis

	Number of days exposed							Number of days of flushing					
Tissue	0	3	7	14	28	42	56*	14	28	42	56	70	84***
Brain	.4	3.6	6.0	8.6	8.4	12.	8.3	7.6	5.8	5.0	3.5	3.4	2.9
	(.1)	(1.1)	(2.1)	(3.9)	(3.7)	(5.9)	(5.2)	(5.6)	(4.7)	(4.1)	(3.5)	(3.4)	(3.1)
Gills	.2	9.0	11.	29.	40.	39.	46.	30.	30.	24.	12.	14.	12.
	(.1)	(8.3)	(13.)	(41.)	(64.)	(82.)	(74.)	(75.)	(72.)	(65.)	(35.)	(46.)	(37.)
Heart	ND(<.1)	5.5	6.8	10.	12.	17.	13.	10.	7.8	6.1	5.3	4.7	2.5
		(.3)	(.5)	(1.0)	(.8)	(1.6)	(1.5)	(1.7)	(1.0)	(.8)	(1.0)	(.8)	(.6)
Liver	1.2	34.	75.	96.	210.	107.	83.	65.	52.	28.	17.	29	22.
	(.1)	(12.)	(42.)	(92.)	(126.)	(86.)	(72.)	(83.)	(60.)	(38.)	(30.)	(58.)	(35.)
Muscle	.1	1.0	1.8	3.6	7.4	7.6	6.5	6.9	4.8	3.4	3.3	2.5	2.0
	(.1)**	(1.8)	(4.5)	(17.)	(24.)	(36.)	(45.)	(65.)	(45.)	(34.)	(44.)	(40.)	(32.)
Whole fish	.1	3.5	7.2	17.	37.	30.	27.	25.	19.	12.	9.3	9.3	7.2***
	(1.5)	(72.)	(201.)	(661.)	(1417.)	(1553.)	(1983.)	(2452.)	(2041.)	(1373.)	(1309.)	(1486.)	(782.)

* The same tissues analyzed from control spot contained no detectable Aroclor 1254 (<.1 to<1.0 ppm depending on sample weight).

** Total micrograms in sample. Sample approximately 26% of total amount of muscle.

*** Other tissues analyzed on day 84 were: Gall bladder 1.9 (.9), gonad .9 (.3), gut 2.5 (7.8), rest of muscle 4.5 (133.), and skin 12. (174.).

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* The same tissues analyzed from control trout contained no detectable Aroclor 1254 (<0.1 to 0.9 ppm depending on sample weight).

** Total micrograms in sample. Sample approximately 26% of total amount of muscle.

*** Other tissues analyzed on day 84 were: Gall bladder 1.9 (.9), gonad .9 (.3), gut 2.5 (7.8), rest of muscle 4.5 (133.), and skin 12. (174.).

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Effects of PCB Administration on Microsomal Enzyme Activity in Pregnant Rabbits

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Polychlorinated biphenyls (PCB's) are industrial chemicals used as plasticizers in paints, resins and plastics, as well as for insulators, heat exchange fluids and a wide variety of other applications (1). PCB residues have been reported in fat samples of various wildlife species, particularly fish and fish-eating birds (1-6)

The limited amount of toxicological data on these compounds has been recently reviewed by Peakall and Lincer (7) and demonstrates the lack of information and effects of PCB's in pregnant animals. This work was designed to study the biochemical and physiological effects of PCB administration on pregnant rabbits and their fetuses.

Methods and Materials

Aroclors* 1221 and 1254 were obtained through the courtesy of Monsanto Company, U.S.A. Twenty-four mature female rabbits, 2500-3000 g body weight were randomized into 6 groups of four animals (2 control and 4 treatment groups) and after mating were dosed daily with either corn oil (c. o.), Aroclor 1221 (1.0 and 10 mg/kg body weight), or Aroclor 1254 (1.0 and 10 mg/kg body weight). Both Aroclors were made up as a 50% (w/v) solution in corn oil.

Twenty-eight days later the does were killed, and their organs removed and weighed. Placentae and fetuses were removed from pregnant does. Tissues were placed on ice for immediate use in enzyme assays and the remainder frozen. Blood samples were taken from the does at the time of death by venapuncture of the abdominal aorta and serum cholesterol determined (8). Liver vitamin A was determined by a colorimetric method using antimony trichloride following saponification in KOH (9). Carboxylesterase activity was determined on liver homogenates using *p*-nitrophenylbutyrate (ONPB) as substrate (10). Protein was determined according to the method of Gornall et al. (11) and enzyme activity was expressed as uMoles substrate hydrolyzed per hour per mg protein at 25°C. Parathion (*O*,*O*-diethyl *O*-4-

*Registered trade names, Monsanto Co., St. Louis, Missouri, U.S.A.

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nitrophenyl phosphorothioate) degradation by liver microsomes was determined by the method of Nakatsugawa et al. (12). Results are expressed as uMoles paraoxon (O,O-diethyl O-4-nitrophenyl phosphate) or p-nitrophenol formed per hour per mg protein. All analyses were done on paraoxon treated microsomes (13). In vitro drug metabolizing activities were determined on a 20,000 G x 20 min supernatant of freshly homogenized liver tissue (10% w/v in 0.25M sucrose). The activities monitored were the N-demethylation of aminopyrine (14) to 4-aminoantipyrine and the aromatic hydroxylation of aniline to p-aminophenol (15). The rates of reaction were linear with respect to protein concentration and incubation time.

Results

Table I summarizes the data on doe body weight gain, conception rate and number of fetuses in the pregnant rabbits.

TABLE I
Effect of Aroclors on body weight gain, conception rate and number of fetuses in rabbits

Group	Number mated	Number pregnant	Average Body Weight Gain ± S.E.M.	Number of Fetuses per litter
Control	8	4	729±62	8.5
Aroclor 1221 1.0 mg/kg b.w.	4	4	605±100	7.25
Aroclor 1221 10 mg/kg b.w.	4	2	661±104	8.5
Aroclor 1254 1.0 mg/kg b.w.	4	3	596±55	6.0
Aroclor 1254 10 mg/kg b.w.	4	4	603±30	6.75

There was no significant difference between controls and treated groups in body weight gain or the number of fetuses per litter. PCB ingestion had no effect on body weight gain, heart, spleen, kidney or brain weights in the dams. Similarly, there was no significant effect of treatment on the fetal liver or the placentae wet weights (expressed as a percent of the

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fetal body weight) or on the fetal body weights. The only significant changes (expressed as percent body weight) were increased dam liver weights from the 10 mg/kg Aroclor 1254 treatment. The results from the serum cholesterol determinations indicate that there was no difference between treatment groups.

The results of the liver vitamin A stores in pregnant rabbits and fetuses are shown in Table II.

TABLE II
The effects of PCB administration on Vitamin A storage in the livers of pregnant rabbits and fetuses

	Vitamin A ug/g liver	Vitamin A ug/whole liver	Vitamin A ug/g liver	Vitamin A ug/whole liver
	DOES		FETUSES	
Control	99.0[1]±19.7 (4)	9107±1538	13.8[1]±1.9 (8)	33.9±5.2
Aroclor 1221 1.0 mg/kg b.w.	114.2 ± 9.3 (4)	10258±1058	18.1*±1.3 (9)	40.8*±3.3
Aroclor 1221 10 mg/kg b.w.	75.9 ± 6.08 (2)	7263± 768	13.2±1.1 (6)	30.7±5.8
Aroclor 1254 1.0 mg/kg b.w.	52.1*± 8.3 (3)	6216± 885	15.8±0.4 (3)	37.1±5.2
Aroclor 1254 10 mg/kg b.w.	56.0*± 8.5 (4)	8367±1410	11.7*±1.7 (8)	25.9*±7.2

[1]All values represent the mean ±S.E.M.
( )Denotes the number of animals used.
*Denotes significant difference from control at 5% level ("t" test).

The concentration of liver vitamin A was lower in animals receiving Aroclor 1254; however, no significant differences were observed in the total amount of vitamin A stored per liver. Aroclor 1221 was without significant effect on vitamin A storage in the maternal liver. Vitamin A storage was increased in fetal liver by Aroclor 1221 (1.0 mg/kg b.w.) and decreased by Aroclor 1254 (10 mg/kg b.w.). The other levels of Aroclor 1221 and 1254 did not significantly alter the vitamin A content in fetal liver.

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There was no effect of PCB administration on protein levels in maternal, and fetal liver homogenates. The protein concentration of placental homogenates was higher only in animals receiving 10 mg/kg b.w. Aroclor 1254.

Carboxylesterase activity in the doe liver homogenates are shown in Table III.

TABLE III
Carboxylesterase activity[1] in liver of pregnant rabbits

GROUP	umoles ONPB hydrolysed per hour per mg protein	mmoles ONPB hydrolysed per hour per g liver	mmoles ONPB hydrolysed per hour per whole liver
Control	345 ± 43 (4)	50.1 ± 8.9	4957 ±1093
Aroclor 1221 1.0 mg/kg b.w.	384 ± 77 (4)	63.6 ±15	5696 ±1436
Aroclor 1221 10 mg/kg b.w.	278 ± 55 (2)	45.7 ± 6.4	4378 ± 745
Aroclor 1254 1.0 mg/kg b.w.	506 ±162 (3)	71.9 ±21	8200 ±1782
Aroclor 1254 10 mg/kg b.w.	580*± 82 (4)	95.6*±15	14038**±1913

[1]All values represent the mean ±S.E.M.
*Indicates significant difference from controls at 5% level ("t" test).
**Indicates significant difference from controls at 1% level ("t" test)
( )Indicates the number of animals.

Aroclor 1254 (10 mg/kg b.w.) administration increased the carboxylesterase activity of maternal liver homogenates There was no change in the carboxylesterase activities in the fetal livers or in the placentae from the treated does as compared with the controls.

The parathion degradation activities of livers from female rabbits are shown in Table IV.

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TABLE IV
Parathion degradation[1] in pregnant rabbits

GROUP	Paraoxon formed per ml homogenate[2]	Paraoxon formed per mg protein[3]	PNP formed per ml homogenate	PNP formed per mg protein
Control	0.031± 0.005 (4)	0.0094± 0.0022	0.026± 0.004	0.0084± 0.0025
Aroclor 1221 1.0 mg/kg b.w.	0.034± 0.005 (4)	0.0091± 0.0016	0.024± 0.006	0.0061± 0.0008
Aroclor 1221 10 mg/kg b.w.	0.028± 0.001 (2)	0.0077± 0.0016	0.018± 0.004	0.0050± 0.0002
Aroclor 1254 1.0 mg/kg b.w.	0.035± 0.004 (3)	0.0077± 0.0013	0.034± 0.004	0.0079 0.0010
Aroclor 1254 10 mg/kg b.w.	0.043± 0.006 (4)	0.0078± 0.0018	0.052*± 0.008	0.0089± 0.0017

[1]Rates are expressed in terms of umoles paraoxon or PNP formed per hour at 25°C. Values represent the mean ±S.E.M.
[2]Microsomal homogenate.
[3]Microsomal protein.
( )Indicates the number of animals.
*Indicates significant difference at 5% level ("t" test).

Control and treated groups were comparable in their ability to degrade parathion to paraoxon. However, the Aroclor 1254 group (10 mg/kg body weight) showed an increased p-nitrophenol formation when considered per ml homogenate but not per mg protein. There was some parathion degradation activity in the microsomes of the placenta and from the fetal livers but it was too low to be determined accurately.

The results for liver aniline hydroxylase and aminopyrine n-demethylase enzyme activities are shown in Table V.

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TABLE V
Liver aniline hydroxylase and aminopyrine n-demethylase activity in pregnant rabbits

GROUP	umoles p-AP[1] per hour per 100 mg protein	umoles p-AP per hour per gram tissue (wet wt.)	umoles 4-AAP[2] per hour per 100 mg protein	umoles 4-AAP per hour per gram tissue (wet wt.)
Control	0.59±0.11 (4)	0.47±0.07 (4)	0.17±0.04 (4)	0.15±0.04 (4)
Aroclor 1221 1.0 mg/kg b.w.	0.40±0.05 (4)	0.36±0.05 (4)	0.15±0.04 (4)	0.14±0.04 (4)
Aroclor 1221 10 mg/kg b.w.	0.59±0.10 (2)	0.31±0.0 (2)	0.192±0.05 (2)	0.092±0.04 (2)
Aroclor 1254 1.0 mg/kg b.w.	0.61±0.10 (3)	0.45±0.04 (3)	0.13±0.01 (3)	0.10±0.01 (3)
Aroclor 1254 10 mg/kg b.w.	0.83±0.10 (4)	0.92*±0.16 (4)	0.52*±0.09 (4)	0.61*±0.15 (4)

[1]p-aminophenol.
[2]4-aminoantipyrine.
*Indicates significant difference at 5% level ("t" test).
( )Indicates number of animals.

There was no difference between control and treated groups when the aniline hydroxylase enzyme activity was expressed in terms of mg protein. However, 10 mg/kg b.w. Aroclor 1254 significantly increased aniline hydroxylase activity when expressed per gram of tissue and increased aminopyrine n-demethylase activity both in terms of activity per 100 mg protein and per weight of tissue.

Discussion

Many pesticides are known to influence reproduction in mammalian species (16, 17). Because of the similarity of PCB's to some of the organochlorine insecticides and the finding of PCB residues in animal species, including man (18), it was of interest to determine the effect of sub-acute administration of two PCB's on reproductive parameters in the rabbit. The results indicate that the administration of Aroclors 1221 and

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1254 for the first 28 days of gestation did not affect implantation, fetal development, fetal growth or litter size at dose levels of 1.0 and 10 mg/kg b.w.[1] The only organ weights observed to be affected were liver weights in the does receiving 10 mg/kg Aroclor 1254. Fetal body weights, liver weights and placenta wet weights were unaffected by PCB administration to pregnant does. Placental transfer of both compounds occurred as evidenced by the detection of residues of the PCB's in the fetuses. The details of these results will be reported in another communication. It has been reported that PCB's fed to rats at 100 mg/kg b.w. per day for 28 days caused elevated serum cholesterol levels (20). However, in our case, PCB administration did not cause any alterations in serum cholesterol levels in pregnant does.

Multigeneration studies in rats fed a dietary regimen of 20 p.p.m. DDT have shown that the liver vitamin A of the pups (21 days of age) was decreased (21). In the present experiment vitamin A storage in the fetal liver was decreased only by the highest dose level of Aroclor 1254. This observation will require further study to assess its significance.

One of the parameters of enzyme induction is increased protein synthesis. The only significant increase in protein levels of homogenates was observed with placental homogenates from rabbits receiving 10 mg/kg b.w. Aroclor 1254; however, no change was observed in the carboxylesterase activity of the placenta. Carboxylesterase activity, used as a indicator of microsomal enzyme activity, was elevated in liver homogenates of does receiving 10 mg/kg b.w. Aroclor 1254. However no change was observed in the carboxylesterase activity of fetal liver homogenates. In vitro, parathion is metabolized to either paraoxon or to p-nitrophenol. Neal (22) has demonstrated that pretreatment of rats with the enzyme inducer 3,4 benzpyrene stimulated the formation of paraoxon to a greater degree than the formation of p-nitrophenol. With phenobarbital pretreatment, about equal stimulation of the two pathways of metabolism occured. In this study the ability of rabbit liver microsomes from does exposed to Aroclor 1254 (10 mg/kg b.w.) to degrade parathion to p-nitrophenol was enhanced while paraoxon formation was not affected.

The drug metabolizing enzymes aniline hydroxylase and aminopyrine n-demethylase were both induced by the administration of 10 mg/kg b.w. Aroclor 1254. This suggests that Aroclor 1254 may increase hydroxylation of steroids in vivo and possibly

[1] In a subsequent study it was found that the administration of Aroclor 1254 at levels of 12.5, 25.0 and 50.0 mg/kg b.w. during the first 28 days of gestation had embryotoxic effects in the rabbit. The details of this study will be published elsewhere (19).

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interfere with reproductive processes. However, in this case, no effects on reproduction were noted.

From these results, it appears that the no effect level of Aroclor 1254 for enzyme induction in the pregnant rabbit is between 1.0 and 10 mg/kg body weight when administered for 28 days during gestation.

Aroclor 1221 did not induce any enzyme activity in the does, foetus or placenta so its no-effect level must be considered higher than that for Aroclor 1254. The important point for both Aroclor 1254 and 1221 is that placental transfer does occur, but does not cause any changes in the biochemical or physiological parameters measured.

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Polychlorinated Biphenyl (Aroclor 1242®): Effects of Uptake on Growth, Nucleic Acids, and Chlorophyll of a Marine Diatom

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Introduction

Polychlorinated biphenyls (PCB's), similar in structure and effects (7) to certain chlorinated hydrocarbon pesticides have been found in ecosystems of Britain (4), northern Europe (5), and in several sections of the United States (3). PCB's employed as plasticizers, dielectrics, and heat transfer fluids have grown in use since the forties in a manner somewhat paralleling DDT. Their seeming ubiquity in the environment and confusion with DDT in chromatographic detection have caused some investigators (2) to suggest that DDT values stated in the literature may be overstated by as much as 90%. Additionally PCB's have been shown to be powerful inducers of hepatic enzymes (8) as well as inhibitors of the carbonic anhydrase system essential to calcium deposition in egg shell production. These factors alone emphasize urgency in the further investigation of PCB distribution in the global environment, primary sources of release and effects on basic food systems of which marine diatoms play an important part. Because previous work (6) had shown Cylindrotheca closterium to be capable of absorbing, concentrating, and metabolizing DDT, similar work was undertaken with the organism and PCB.

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Methods

Sixteen one liter erhlenmeyer flasks, each containing 200 ml. of culture media were inoculated with the diatom C. closterium, Reimann and Lewin. Four flasks served as controls, four for each level of PCB (Aroclor 1242) treatment, and four as acetone controls since acetone was the vehicle for the toxicant.

Cell counts were made at the conclusion of a two week incubation period. The contents were then centrifuged, the resulting pellet lyophilized, weighed and assayed for PCB, nucleic acids, and chlorophyll spectra lipids.

PCB (Aroclor 1242) was extracted from the dry pellet with 1 ml. of nanograde acetone. A suitable aliquot was injected into the gas chromatograph for analysis. Two column systems were employed: (1) a six foot X 1/4 inch O.D. glass column packed with 4% SE-30-2% QF-1 on Chromoport XXX and, (2) a six foot X 1.4 inch O.D. glass column packed with 1.5% OV-17-1.95% QF-1 on Chromoport XXX. The operating parameters of the gas Chromatograph were as follows:

Inlet temperature	235°C
Column temperature	200°C
Detector temperature	350°C
Carrier gas flow	60 cc/min

Identification of Aroclor 1242 was accomplished by measuring relative retention times of the five major peaks along with relative peak heights. Quantification was accomplished by comparison of the area under the curve of the five late eluters with the standard Aroclor.

Verification of the peak identities by thin layer chromatography positively identified Aroclor 1242 as the major PCB component of the sample. Some PCB materials not common to the known (Aroclor 1242) mixture were isolated. These materials were, in all cases, early eluters, and possibly metabolic products of Aroclor 1242.

RNA and DNA were assayed using a modification of Agranoff's procedure (1). Chlorophylls and related materials were chloroform: methanol (2:1) extracted and optical density measured at 670 microns. The chlorophyll index which will be referred to is actually the maximum optical density of all chloroform methanol extractible materials read at the maximum absorbance for chlorophyll.

Results and Discussion

As shown in Table 1, PCB was taken up by C. closterium and concentrated up to 1100 times above levels added to culture media. The higher media level of PCB (.1 ppm) sharply inhibited growth as evidenced

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by harvest weights and cell counts. The .1 ppm dosage also significantly reduced RNA synthesis and the chlorophyll index but apparently had no effect on DNA levels. PCB at .01 ppm in the media did not seem to adversely affect growth or seriously alter nucleic acid levels or chlorophyll production.

Conclusion

Cylindrotheca closterium absorbed and concentrated the polychlorinated biphenyl (Aroclor 1242) 900 to 1000 times above the level in sea water. At .1 ppm concentration in sea water, PCB's inhibited growth and diminished levels of RNA and chlorophyll of this marine diatom.

TABLE 1

Harvest Weight, Cell Counts, Nucleic Acid Levels, and Chlorophyll Index of Cylindrotheca closterium Cultures Exposed to Polychlorinated Biphenyls

Treatments	Harvest Weight (mg)	Final Cell Count ($X\ 10^4$)	Chlorophyll Index (OD @ 670)	RNA (mg/mg)	PCB Levels (ppm)
PCB .1 ppm	11.5**	16.5**	.27**	.015**	109.2**
PCB .01 ppm	38.1	113.7	1.26	.033*	4.7*
Control	30.9	116.8	1.52	.039	0
Acetone Control	33.2	98.5	1.37	.040	0

* Significantly different from control at .05 level

** Significantly different from control at .01 level

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ENA (µg/mg)	PCB Levels (ppm)
.015**	109.2**
.033*	4.7*
.039	0
.040	0

.05 level

.01 level

Acknowledgement

This research was partially supported by NIH General Research Support Grant 5420.

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Retention Times and Electron-Capture Detector Responses of Some Individual Chlorobiphenyls

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Retention times and electron-capture detector responses of 23 chlorobiphenyls are reported in this paper. Polychlorinated biphenyls (PCB) are global pollutants (1) and gas chromatography has been used to determine PCB in the environment (2,3,4). Only commercial PCB preparations, containing mixtures of chlorinated biphenyls are commonly available as standards and more knowledge of the behaviour of the individual chlorinated biphenyls during gas chromatography is desirable. Retention times and electron-capture detector responses have been reported for mono- and dichlorobiphenyls (5,6). An excellent separation method for mono-, and some di- and trichlorobiphenyls by gas chromatography on polyphenyl thioethers has been patented (7). As a general rule, chlorine substitution in position 2 shortens and vicinal disubstitution lengthens the retention time. The reported relative electron-capture detector responses are 1, 0.2, 2.3, 7.6, 5.8, and 3.5 for 4-, 3-, 2-mono-, 4,4'-, 3,3'-, and 2,2'-dichlorobiphenyl, respectively (6).

Experimental

The preparation and characterization of the individual chlorinated biphenyls is described elsewhere (8). A Packard A7901 gas chromatograph with a glass column (6 ft x 4 mm), containing 4% SE-30 on acid-washed Chromosorb W, 100/120 mesh, operated at 200°C, was used. Carrier gas was nitrogen at a flow rate of 60 ml/min. Injector and detector were kept at 210°C. D.C. voltage in the electron-capture detector was 95V and meter sensitivity was 1×10^{-8} Amp. Solutions of chlorinated biphenyls in pesticide-grade hexane were used, a 25:75 v/v mixture of pesticide-grade benzene and hexane was required to dissolve decachlorobiphenyl. Concentration of chlorinated biphenyls in solutions injected on the column was 1 - 260 µg/ml, and volumes from 2 to 5 µl were injected to obtain peak heights of 30-70% of the full scale pen deflection. Areas under the peaks were measured by a disc integrator. Every compound was injected at least four times to check the linearity of the detector response. p,p'-DDE was used as the standard. Total peak area was used as the

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detector response for the two commercial polychlorinated biphenyl preparations, Aroclor 1254 and Aroclor 1260.

Results and Discussion

Relative retention times and electron-capture detector responses of chlorinated biphenyls and of Aroclor 1254 and 1260 are presented in Table 1. Both the retention time and the detector response depend on the number of chlorine atoms in the molecule and on the substitution patterns. Chlorine substitution in positions 2 and 6 shortens, and vicinal substitution lengthens, the retention time also in tri- and tetrachlorobiphenyls. In some instances these effects overshadow the effect of the number of chlorines in the molecule which increases the retention time.

The detector response increases strongly with increasing number of chlorine atoms in the molecule. Thus the response of decachlorobiphenyl is 500 times stronger than the response of 4-chlorobiphenyl. Most of the increase occurs in the mono- to trichlorobiphenyl range and the response increases only by a factor of approximately 2-3 between tetra- (with the exception of 2,2',4,4'- and 2,2',6,6'-tetrachlorobiphenyl) and decachlorobiphenyl. Chlorine substitution in positions 2 and 6 decreases and vicinal substitution, particularly in positions 3 and 4, increases the electron-capture detector response.

Polychlorinated biphenyls found in wildlife are of the Aroclor 1254 or 1260 type (2,3,4). These Aroclors give under our conditions 14 and 16 peaks with relative retention times from 0.48 to 3.28 and from 0.72 to 6.80, respectively. Additional chlorinated biphenyls will have to be prepared to be able to identify the components of Aroclor 1254 and 1260. The presented data do indicate, however, that the electron-capture detector response of all chlorinated biphenyls with 4-9 atoms of chlorine per molecule is not likely to exceed the value of 1.6 relative to p,p'-DDE under the described conditions.

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TABLE 1

Retention time and electron-capture detector response of chlorobiphenyls

Compound	Relative retention time (p,p'-DDE=1.00)	Relative response per ng ± standard deviation (p,p'-DDE=1.00)
4-chlorobiphenyl	0.17	0.0033±0.00005
2- "	0.11	0.0030±0.00004
3- "	0.14	0.0006±0.00001
4,4'-dichlorobiphenyl	0.30	0.0152±0.0002
3,3'- "	0.25	0.0155±0.0001
2,2'- "	0.15	0.0131±0.0001
3,4- "	0.28	0.0388±0.0002
2,4- "	0.19	0.0450±0.0004
2,6- "	0.16	0.0815±0.0049
2,4,4'-trichlorobiphenyl	0.39	0.298±0.010
2,4,6- "	0.24	0.276±0.006
2,2',4,4'-tetrachlorobiphenyl	0.51	0.206±0.003
3,3',4,4'- "	0.96	0.770±0.027
2,2',6,6'- "	0.33	0.0403±0.0016
3,3',5,5'- "	0.70	0.625±0.045
2,3,4,5- "	0.69	0.715±0.010
2,3,5,6- "	0.51	0.505±0.010
2,3,4,5,6-pentachlorobiphenyl	1.00	1.30±0.017
2,2',4,4',6,6'-hexachlorobiphenyl	0.78	0.545±0.029
3,3',4,4',5,5'- "	2.98	1.15±0.064
2,2',3,3',4,4',6,6'-octachlorobiphenyl	2.62	1.58±0.020
2,2',3,3',5,5',6,6'-octachlorobiphenyl	2.45	1.53±0.076
Decachlorobiphenyl	8.20	1.61±0.086
Aroclor 1254		0.910±0.008
Aroclor 1260		1.35±0.010

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Thin-Layer Chromatography of Polychlorinated Biphenyls

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INTRODUCTION

Polychlorinated biphenyls (PCB's) have been recognized as being industrial pollutants of the environment. Their presence in the form of residues in wildlife samples was first reported in Sweden in 1966 (1) and later in several other European countries and in North America.

PCB's have a tendency to accumulate in fatty animal tissues as for instance p,p'-DDE. They are not pesticides, but can be determined according to the usual analytical methods for detecting organochlorine insecticides. Until now only gas chromatography has been reported in the literature as a technique for separating the various components of the complicated mixtures represented by the PCB's (2,3,4,5).

Thin-layer chromatography is a useful technique in pesticide residue analysis for the qualitative confirmation of results obtained by means of gas chromatography. When an often used TLC-system like aluminum oxide/hexane is applied , however, very little information is obtained about the composition of the PCB residue. Only a diffuse and rather long-drawn spot is found with an approximate R_f-value between 0.6 and 0.8.

So far no TLC system was available that is particularly suitable for satisfactory separation of individual PCB compounds. Reversed-phase partition TLC (utilizing a non polar stationary phase and a polar mobile phase) is used for resolving closely related lipophilic materials, such as homologous series of fatty acids. We have found that this technique can be applied in separating closely related components in PCB mixtures. Details of the method, which was worked out for this purpose, are given below.

EXPERIMENTAL

1. Preparation of plates

A quantity of 25g "Kieselguhr G"(Merck no.8129) was mixed with 60 ml water and stirred until a homogeneous slurry was obtained. Any remaining air bubbles were removed by applying vacuum. The slurry was transferred to a Desaga applicator and 5 glass plates (20 x20 cm) were coated in the usual way (layer thickness 0.25mm). The plates were air-dried overnight and finally heated for one hour at 105°C. The plates were cooled and left uncovered (not in a desiccator).

A flat tray was filled with petroleum ether (b.p. 40-60°C)

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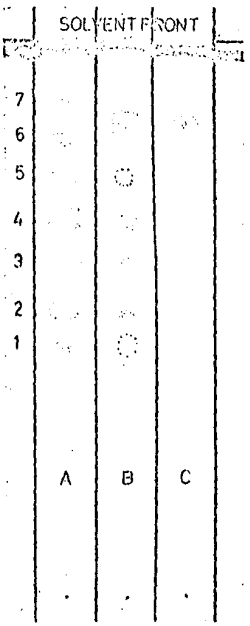

Figure 1

Thin layer chromatograms of, "A" technical PCB mixture, "B" extract of owl liver, and "C" p,p'-DDE. Kieselguhr impregnated with liquid paraffin, developed 3x with a mixture of acetonitrile, acetone, methanol, and water (40:18:40:2), and visualized with silver nitrate and U.V.

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containing 8 vol.% liquid paraffin. A coated plate was carefully soaked in this solution until the adsorbent layer was saturated all over with the solvent, which usually took 1-2 minutes. Then the plate was carefully removed from the solution. After air-drying and storage for at least 24 hours, the plate was ready for use. Plates thus prepared could be kept for a considerable time, at any rate for several weeks.

2. Spotting and development

Hexane solutions of standards and residues were spotted along the origin of a plate. The best results were obtained when a quantity of not more than 20µl was spotted.

A glass developing tank, lined with filter paper, was filled with a solvent mixture of 40 ml acetonitrile + 18 ml acetone + 40 ml methanol + 2 ml water. This mixture had previously been saturated with liquid paraffin by shaking in a separatory funnel. The tank was closed. When the solvent had reached the top of the liner, a plate was transferred to the tank. The solvent front was allowed to travel 14 cm above the origin line of the plate. After being developed the plate was air-dried, then the developing procedure was repeated two additional times. The plate was dried after each development. Three developments gave the best resolutions at moderate room temperature(± 20°C). At elevated temperatures (above + 25°C) it was found that R_f values for PCB-spots tended to be too high. In these cases better results were obtained with only two developments.

3. Visualization

After developing the plate was air-dried until the solvent had entirely evaporated. The plate was sprayed with about 10 ml of a silver nitrate spray, prepared by dissolving 1.7 g silver nitrate in 200 ml ethanol (96%). The plate was irradiated with UV-light (Philips TUV 15 watt) at a distance of about 15 cm. Black spots on a white background usually appeared within 20 min.

RESULTS AND DISCUSSION

Figure 1 shows the results obtained with the method described. A is a chromatogram of 15 micrograms of Phenochlor DP6 a commercial PCB preparation containing 60% chlorine. Other brand names for industrial preparations are Aroclor 1260 and Clophen A 60.

Residue studies in the Netherlands (2) have shown that PCB residues in fish and birds have a GLC-peak pattern very similar to that of the mixtures mentioned above. B is an example of a residue found in a wildlife sample, the liver of a long-eared owl from the northern part of The Netherlands. For the analysis the liver was ground with sand and sodium sulfate and extracted with hexane. The hexane extract was cleaned up by chromatography on two small alumina columns, as described by Holden and Marsden (6).

C is a p,p'-DDE standard which was spotted for comparison.

Fig. 1 demonstrates that also by TLC a strong resemblance is found between the spot patterns of the residue and the technical PCB mixture.

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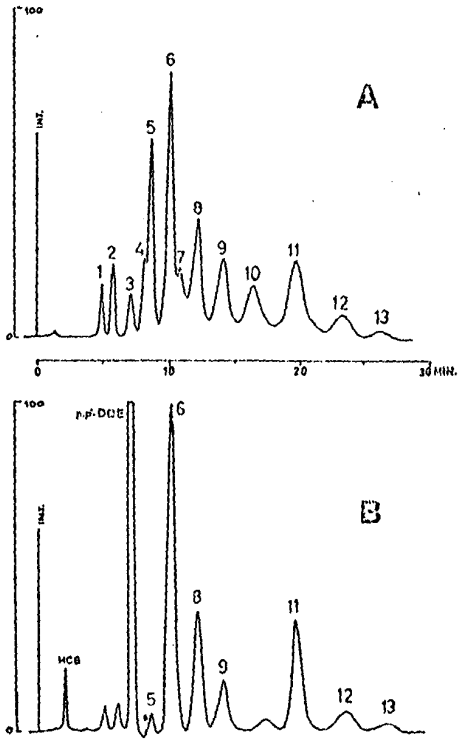

FIG. 2

Gas chromatograms of "A" Phenochlor DP6 (0.6 µg) and "B" hexane extract of owl liver. Instrumental conditions are given in Table 1.

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Gas chromatograms of Phenochlor DP6 and the liver extract are shown in Figs. 2A and B. A dohrmann microcoulometer, equipped with a silver cell, was used as a detection system. Further instrumental conditions and relative retentions of the peaks in Fig. 2A are summarized in Table 1.

The PCB-residue (estimated by microcoulometry) in the liver sample was 350 ppm. Besides this residue, hexachlorobenzene (6.5 ppm), heptachlorepoxide (3.6ppm) and p,p'-DDE (290 ppm) were also detected. The sample was selected for demonstration purposes; the detected residues are high and cannot be regarded as general background residues in Dutch birds.

TABLE 1

Relative retentions (dieldrin = 1.00) of PCB-peaks 1-13 in Fig. 2 A.

Peak No.	Rel. retention[a]
1	0.71
2	0.84
3	1.03
4	1.18
5	1.23
6	1.47
7	1.59
8	1.73
9	2.01
10	2.33
11	2.80
12	3.33
13	3.76

Instrument: Microtek MT 220. Column: glass, 6' x1/4").D., packed with 3% OV-1 on Gas Chrom Q, 80-100 Mesh. Operating temperatures: column 200°C; injector 220°C; transfer line (Teflon coated aluminum) 220°C Detection system: Dohrmann C 250 A microcoulometer, with oxidative pyrolysis furnace and T 300 S titration cell. Sensitivity: 200 ohms. Gain: low (200). Recorder sensitivity: 1 mv. Chart speed: 1 cm/min.

Peak numbers 5, 6, 8, 9, 11, 12, and 13 in Fig. 2 B have the same retention times as the corresponding peaks in Fig. 2 A.

Previous investigations, carried out at our institute with a gas chromatograph - mass spectrometer combination (2), have revealed that in the chromatogram of the technical mixture containing 60% chlorine, peaks 1 and 2 are produced by pentachlorobiphenyls, peaks 3, 4, 5, 6, and 8 by hexachlorobiphenyls, peaks 7, 9, 10, 11, and 12 by heptachlorobiphenyls, and finally "peak" 13 by octachlorobiphenyl(s).

In order to ascertain the relation between spots A 1 to A 7 on the plate (Fig. 1) and peaks 1-13 in Fig. 2 A, several aliquots of Phenochlor DP6 solution in hexane were spotted along the origin

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of a TLC plate. After developing, as described before, small vertical areas on the plate were treated with silver nitrate and irradiated to locate the various rows of spots. Horizontal zones corresponding to spots 1-7 were scratched out. The absorbent fractions obtained were eluted and analysed by gas chromatography under conditions as stated in Table 1. The results are reported in Table 2.

This table shows that spots with lower R_f-values generally correspond to peaks with higher retention times, or in other words to PCB's with a higher degree of chlorine substitution (as follows from the already mentioned mass spectrometric observation). Spot A 1 corresponds to peak 13, but also to a peak eluting very late with relative retention 5.6 (dieldrin = 1.00). This peak is normally not visible in the gas chromatogram, unless more concentrated solutions are injected. The identity of this peak has not yet been investigated by means of mass spectrometry.

TABLE 2
Relationship between spots obtained by thin-layer chromatography (Fig. 1 A) and peaks obtained by gas chromatography (Fig. 2 A) of Phenochlor DP6

spot no.	corresponding peak numbers
1	13
2	11
3	6, 9, 12
4	7, 8, 10
5	2, 4, 5
6	3, 6
7	1, 3

When observing the peaks and spots found with the liver extract it can be seen that there is a relationship similar to that found with the technical PCB mixture (Table 2).

Of the chlorinated pesticides that are frequently detected in wildlife samples only the fungicide hexachlorobenzene(HCB) interferes with one of the PCB spots (no.3). In the liver sample interference is negligible, since the HCB residue is very low compared with the PCB residue. p,p'-DDT, p,p'-DDE, p,p'-TDE, dieldrin, heptachlorepoxide, and lindane all migrate faster than PCB spot no. 7 (Fig. 1); most of these compounds are found quite close to the solvent front.

The lowest detectable amounts obtained with the reported TLC-system are of the order of 100 ng of single compounds like DDE and HCB, and a few micrograms for PCB mixtures such as Phenochlor DP6.

In summary it can be stated that the reversed phase partition TLC system described was found satisfactory for the separation of the components of a PCB mixture and can be used in combination with gas chromatography for the study of the composition of PCB residues.

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