Table III.5.3 (from ref. 119)

Table 6.41 INCUBATION TIMES AND HATCHABILITIES OF COHO SALMON (Oncorhynchus kisutch) EGGS, AND SURVIVAL AND SIZES OF ALEVINS 4 WEEKS AFTER HATCHING, AFTER EXPOSURE TO AROCLOR 1254 FOR 6 AND 2 WEEKS AT 12-14C

PCB (ppb)	Incubation Time (degree-days)	Hatchability (%)	Alevin Survival (%)	Mean alevin size Length (mm)	Weight (g)
		Exposure for 6 weeks (2 before and 4 after hatching)			
0	511	95.5	91.0	36.0	0.30
5	485	88.0	75.5	34.7	0.30
10	485	78.5	64.0	34.4	0.30
15	-	38.0[1]	24.0	31.9 (12)[2]	0.27
25	459	96.5	6.8	29.8 (4)	0.28
55	462	63.0	6.5	28.3 (3)	0.24
		Exposure for 2 weeks (to 2 days before hatching)			
0	503	93.0	93.0	35.3	0.30
5	475	95.5	69.5	35.2	0.30
10	490	90.0	88.0	34.7	0.30
15	490	91.5	83.5	33.3	0.26
25	481	97.5	74.5	34.5	0.29
55	488	66.0	39.5	33.7 (15)	0.26

[1] A fungus caused high mortality.

[2] Numerals in parentheses are numbers measured; 20 were used in each of other tests.

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of Aroclor 1254; effects were smaller with other Aroclors (Figure III.5.2). These sublethal effects were associated with whole-body residues of PCB in the range 1-5 ppm (Figures III.5.1, III.5.2). Mayer et al. also found that incorporation of Aroclor 1248 into the diets of lake trout at concentrations in the range 0·2 - 6 ppm suppressed growth and serum cortisol levels, but stimulated thyroid activity during the first 160 days of exposure (73, 96).

Walker (73) also reported unpublished experiments in which lake trout exposed to PCBs in the diet developed lesions in the gills and liver during a 6 - 9 month exposure period. Nonspecific degeneration of liver parenchyma and cytoplasmic vacuolation of liver cells were reported in addition to pleomorphism. Spot exposed for two weeks or longer to 5 μg/l of Aroclor 1254 showed fatty changes in their livers, characterized by the presence of large vacuoles within hepatocytes and disorientation of liver cord distribution (105). Affected fish usually developed ragged fins and lesions on the body (111).

Pinfish sublethally poisoned by exposure for 14-45 days to 5 μg/l of Aroclor 1254 similarly developed fungus-like lesions on the body, especially around the mouth, where hemorrhaging also occurred (111). Pinfish similarly exposed to 32 μg/l of Aroclor 1016 suffered progressive erosion of scales, skin and finally, flesh in front of the dorsal fin. These fish also showed several changes in liver and pancreatic tissues. Normal liver cord orientation was altered and severe vacuolation developed in the pancreatic exocrine tissue surrounding the portal veins (100).

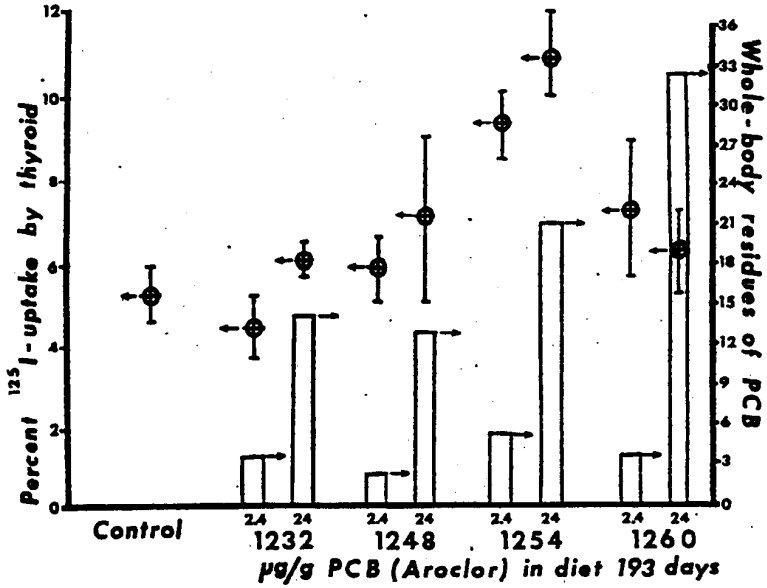

Figure 3. Whole-body residues and thyroidal uptake of ^{125}I in channel catfish fed diets containing Aroclors 1232, 1248, 1253, and 1260 for 193 days (ref. 40).

Figure III.5.1 (from ref. 73)

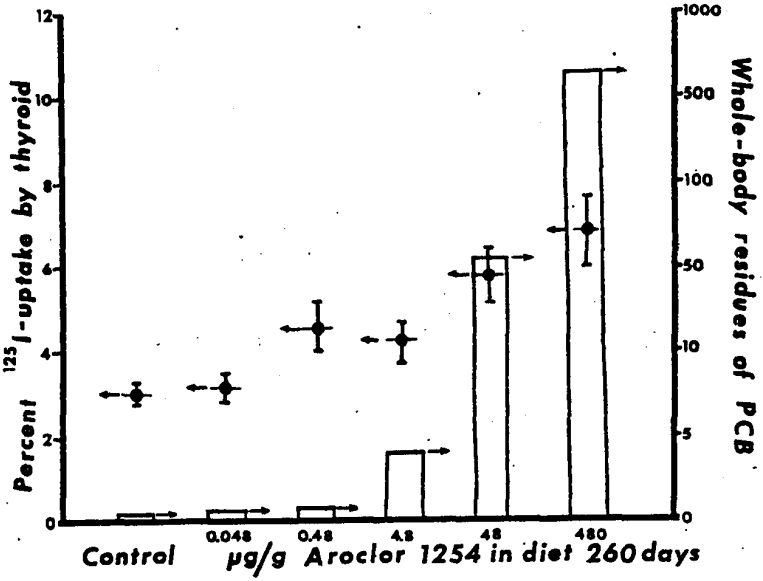

Figure 2. Whole-body residues and thyroidal uptake of ^{125}I in coho salmon fed diets containing Aroclor 1254 for 260 days (ref. 40).

Figure III.5.2 (from ref. 73)

"Fin rot syndrome" has been induced in the laboratory in spot exposed to 3-5 µg/l of Aroclor 1254 (106). The syndrome appears identical to that observed in wild fish (croakers and spot) which died under conditions of warm weather and oxygen depletion in Escambia Bay, Florida (106).

Growth of juvenile coho salmon was significantly reduced (from 1·0 percent of body weight per day to 0·7 percent) when they were fed a diet containing 10 ppm of a mixture of tetra- and hexa-chlorobiphenyls for 165 days (121).

Johansson et al. (122) found that sublethal doses of Clophen A 50 caused metabolic disturbances in brown trout (Salmo trutta). The experimental trout were given two doses (intramuscular injection or capsules directly into the stomach) of 5 mg/kg body weight on days one and five. Analyses were made on day 43 and, after 116 days starvation, on day 203. While the PCB-treated trout seemed to be in good condition after both 43 and 203 days, metabolic changes had taken place. Among the differences on day 43, the treated trout showed anemia, hyperglycemia and altered cholesterol metabolism. After starvation and refeeding, the PCB-treated trout differed from the controls by increased body-weight, a more pronounced decrease in liver triglycerides and muscle glycogen, and no hyperglycemic response.

III.5.4 Effects on ATPase and Osmoregulation

The effect of polychlorinated biphenyls on the ATPase enzyme system was examined in tissues of the bluegill sunfish (Lepomis machrochirus) by Yap et al. (123). ATPase activity was determined in homogenized tissue fractions that contained mitochondria plus nerve endings. Three Aroclors, 1221, 1254, and

1268, inhibited MG^{2+} and Na^{+}-K^{+} ATPases in concentrations less than 1 ppm, with the most pronounced inhibition on Mg^{2+} ATPase of muscle (see Table III.5.4) (123). Cutkomp et al. (124) also found the maximum effects in vitro were on oligomycin-insensitive* Mg^{2+} ATPase, with greatest inhibition in muscle. The effectiveness of Aroclor 1242 on oligomycin-insensitive and oligomycin-sensitive Mg^{2+} ATPase from bluegill muscle was compared at 50 percent inhibition, with values of 0.6 ppm and 2.0 ppm, respectively (124).

In general, the PCBs do not reveal any obvious relationships between chlorination and inhibitory effects. The action of Aroclor 1268 on Mg^{2+} ATPase from different tissues ranged from inhibition (muscle) to activation (brain, liver, and kidney), dependent upon concentration (124, 125). Aroclor 1242 inhibited Na^{+}-K^{+} ATPase from fish brain homogenates but about five times the concentration was required as compared to inhibition of mitochondrial Mg^{2+} ATPase. Mitochondrial Mg^{2+} ATPase was more sensitive to Aroclor 1242 in vivo than non-mitochondrial Mg^{2+} ATPase, the reverse of in vitro findings with fathead minnows (124). Thus, although results are more accurate in vitro than in vivo, both approaches seem desirable. Of the Aroclors tested, those in the intermediate range of chlorination appeared to have the greatest inhibitory effect on the ATPase system, especially Aroclor 1242. Data on inhibition of ATPase from brain, liver and kidney of fathead minnows (Pimephales promelas) are given in Tables III.5.5 and III.5.6 (126). Koch et al. (126) feel that there is sufficient evidence to indicate ATPase inhibition may be the

*Oligomycin-insensitive--non-mitrochondrial.

Table III.5.4 (from ref. 123)

INHIBITION OF Mg^{2+} ATPase and Na^{+}-K^{+} ATPase FROM TISSUES OF BLUEGILL FISH

Concentration (p.p.m.)	Percentage Inhibition							
	'Aroclor 1221'		'Aroclor 1254'		'Aroclor 1268'		'Aroclor 5460'	
	Mg^{2+}	Na^{+}-K^{+}	Mg^{2+}	Na^{+}-K^{+}	Mg^{2+}	Na^{2+}-K^{+}	Mg^{2+}	Na^{+}-K^{+}
Brain								
0.03	0.3	4.6	4.2	16.9	0.4	29.4	2.3	26.9
0.33	8.4	10.5	0.7	19.1	+1.5	27.6	2.9	26.3
3.33	7.2	20.4	27.7	41.5	+14.0	43.6	2.5	38.9
10.00	36.0	46.4	57.9	45.5	8.3	39.4	11.2	42.7
Kidney								
0.33	7.4	15.1	1.3	35.2	0.0	25.4	0.0	22.3
3.33	5.0	24.3	33.4	45.9	+7.9	40.1	4.5	25.2
10.00	42.8	43.3	60.1	51.7	+4.8	48.0	+1.4	33.8
Liver								
0.03	2.2		3.9		—		—	
0.33	2.6		19.4		+5.3		6.6	
3.33	2.9		24.3		+5.1		9.3	
10.00	35.8		53.0		+2.4		15.8	
16.70	45.9		74.1		6.2		—	
Muscle								
0.03	18.7		20.6		37.2		31.9	
0.33	29.9		30.6		47.1		42.9	
3.33	65.4		73.1		60.3		—	
10.00	80.4		84.3		67.5		—	

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Table III.5.5 (from ref. 126)

INHIBITION OF ACTIVITY OF ATPases BY A 4 MONTH EXPOSURE TO AROCLOR 1242 IN FAT HEAD MINNOW TISSUES

Concentration (ppb)	Percent Inhibition*								
	Brain			Kidney			Liver		
	$Na^+ - K^+$	Oligomycin** Sens. Mg^{2+}	Oligomycin Insens. Mg^{2+}	$Na^+ - K^+$	Oligomycin Sens. Mg^{2+}	Oligomycin Insens. Mg^{2+}	$Na^+ - K^+$	Oligomycin Sens. Mg^{2+}	Oligomycin Insens. Mg^{2+}
0.93	21.1	28.1	15.0	20.5	56.0	22.7	+41.6	0.8	7.9
2.8	4.9	53.2	2.3	36.2	75.4	20.7	+40.7	40.5	3.7
8.3	2.9	43.6	+0.7	18.3	45.2	35.0	42.4	40.7	15.5
Control***	34.3	5.2	12.6	39.3	9.0	18.4	3.6	7.6	8.2
Sp. Act. ±S.E.	±1.8	±1.1	±0.45	±2.02	±1.85	±1.35	±0.61	±1.6	±1.9

* (+) Values represent increased enzyme activity.

** Sens. (sensitive) Insens. (insensitive).

*** Specific activity calculated as μmoles Pi mg^{-1} protein hr^{-1}. Each value represents the mean from three separate determinations.

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Table III.5.6 (from ref. 126)

INHIBITION OF ACTIVITY OF ATPases BY A 4 MONTH EXPOSURE TO AROCLOR 1254 IN FAT HEAD MINNOW TISSUES

Concentration (ppb)	Percent Inhibition*								
	Brain			Kidney			Liver		
		Oligomycin**			Oligomycin			Oligomycin	
	$Na^+ - K^+$	Sens. Mg^{2+}	Insens. Mg^{2+}	$Na^+ - K^+$	Sens. Mg^{2+}	Insens. Mg^{2+}	$Na^+ - K^+$	Sens. Mg^{2+}	Insens. Mg^{2+}
0.31	+20.3	31.3	+21.8	+20.0	+53.7	+14.3	--	--	--
0.93	+11.2	35.2	+ 9.8	14.3	+ 7.3	+ 4.9	+20.8	+37.8	+11.4
2.8	+ 3.2	35.8	+ 5.5	5.1	3.3	+22.5	1.0	20.3	20.8
8.3	5.2	7.5	2.2	30.7	+ 4.2	+31.6	4.4	30.7	+ 2.2
Control***	32.6	5.2	12.5	31.4	7.9	12.6	5.9	7.9	9.5
Sp. Act. ±S.E.	±1.95	±0.34	±0.68	±2.45	±2.05	±3.85	±2.0	±2.31	±3.61

* (+) Values represent enzyme activation.
** Sens. (sensitivity) Insens. (insensitive).
*** Specific activity calculated as μ moles Pi mg^{-1} protein hr^{-1}. Each value represents the mean from three separate determinations.

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specific action of PCB in fish tissues. While kidney tissue appeared most sensitive to Aroclor 1242, both liver and kidney had undergone considerable degradation by Aroclors 1242 and 1254 when observed during dissection (126).

Aroclor 1221 affected the ability of the killifish to osmoregulate, since both osmolality and Na^+ concentration increased toward that of sea water (127). Since Na^+-K^+ ATPase is believed to be involved in the process of sodium transport across cell membranes and to function in osmoregulation in marine teleosts, Aroclor 1221 probably acts to disrupt the Na^+-K^+ ATPase system. Serum osmolality and K^+ concentrations were increased well above normal in fish blood sampled before the fish died (127).

III.5.5 Effects of PCDFs on fish

Zitko and Choi (128) fed a diet containing about 20 ppm of PCDFs (2.7 ug/g di-, 5.7 ug/g tri-, 2.8 ug/g tetra- , and 9.1 ug/g octa-chlorodibenzofuran) to juvenile Atlantic salmon. This level of dietary exposure proved only moderately toxic, the median time to death being 120 days. For comparison, coho salmon died after 260 days when fed a diet containing about 100 ppm of PCBs (14.5 mg/kg body weight per day of Aroclor 1254) (96, 121). This indicates that PCDFs are somewhat more toxic than PCBs to fish, but not dramatically so as in the case of birds and mammals (see below).

III.6 Effects on birds

Effects of PCBs on birds were reviewed in 1972 by Vos (129) and most recently by Standell (130).

III.6.1 Lethal concentrations in diet: subacute and semichronic studies

The subacute toxicity of six Aroclor mixtures was studied in 2-week-old chicks of four species of wild birds (mallard, pheasant, bobwhite quail, and Japanese quail) (131). The experimental results were expressed as LC_{50}s based on 5-day feeding (Table III.6.1). In most cases Aroclor 1260 was significantly more toxic than the less chlorinated mixtures. The bobwhite quail was the most sensitive of the four species tested, but even for this species the LC_{50}s were quite high (Table III.6.1).

Numerous studies have been reported of the toxicity of PCBs to young chickens. Five such studies are summarized in Table III.6.2 (from ref. 129). In a feeding study with 400 ppm Aroclor 1260, only 15% mortality was noted in 60 days (137); in the same conditions Clophen A60 and Phenoclor DP-60 were much more toxic (see next section).

In a study with 10-day old chickens, using Aroclor 1248, mortality after 25 days was 16/30 at 50 ppm in the diet, 4/20 at 40 ppm, 1/30 at

Table III.6.1 (from ref. 131)

Toxicity Comparisons of Aroclors and Four Organochlorine Pesticides in Diets of 2-Week-Old Birds (Heath *et al.*, 1970)

Chemical	LC_{50}* with 95% confidence limits parenthesized			
	Mallard	Pheasant	Bobwhite Quail	Japanese Quail
Aroclor 1232	—	3150 (2625–3950)	3000 (2575–3500)	>5060
Aroclor 1242	3180 (2010–3880)	2080 (1840–2350)	2100 (1705–2610)	>5000
Aroclor 1248	2795 (2205–3420)	1310 (1165–1480)	1175 (965–1140)	4845 (4375–5410)
Aroclor 1254	2700 (2160–3310)	1090 (905–1230)	805 (410–810)	2900 (2600–3210)
Aroclor 1260	1975 (1305–2750)	1090 (1105–1435)	735 (575–945)	2185 (1915–2480)
Aroclor 1262	3010 (2400–3835)	1235 (1085–1405)	870 (700–1070)	2290 (2030–2575)
DDE	3570 (2810–4670)	840 (720–970)	825 (695–950)	1455 (1119–1656)
DDT	1870 (1500–2370)	310 (255–377)	610 (515–725)	370 (470–590)
Dieldrin	200 (160–240)	55 (49–61)	39 (33–45)	56 (51–61)
Endrin	22 (17–31)	14 (10.5–17.5)	15 (10.5–24)	15 (12.5–17)

* LC_{50}: ppm chemical in *ad libitum* diet expected to produce 50% mortality in 5 days, 5 days toxic diet followed by 3 days of untreated diet. Each determination based on 60 birds.

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Table III.6.2 (from ref. 129)

Semichronic Oral Toxicity Studies of PCB Preparations.

Preparation	Animal	Treatment	Mortality	Liver effects	Other effects	References
Aroclor 42% Cl	Chicken	100, 200, 400, and and 1000 ppm in diet for 4 weeks	0, 0, 50, and 90% respectively	Enlargement; damage at the higher levels	Edema formation from 200 ppm; at high levels internal haemorrhage and tubular dilatation in kidneys	132
Aroclor 42% Cl	Chicken	200 and 400 ppm in diet for 3 weeks	0 and 12% respectively		Pronounced edema at 400 ppm; enlarged kidneys; small spleen; defeathering and dermatitis	133
48% Cl KC-400	Chicken	1, 5, 10, 25, 50, 100, 300, 600, 1200, 2400, and 4800 ppm in diet for 20 days	0% from 1 to 100 ppm; 100% from the 100 ppm level		Edema formation 100 ppm level	134
Aroclor 48% Cl	Chicken	10, 20, 30, 50, 100, and 150 ppm in diet for 4.5–5 weeks	After 3 weeks: 0, 0, 30, 30 and 20%; at the end 0, 0, 80, 60, and 80% respectively	Enlargement	General edema and depression of the secondary sexual characteristics from the 30 ppm level	135
Aroclor 54% Cl	Chicken	250 and 130 ppm in diet for 6 to 13 weeks	250 ppm 100% between 3 and 10 weeks; 500 ppm some mortality at the end		500 ppm at end: comb weights 30-fold and testes weights 2-fold lower than controls	136

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30 ppm and 0/10 at 10 ppm. Only 2-4 out of 10 chicks survived on diets containing 100 and 150 ppm (138). In a comparative study with Aroclors 1242, 1254 and 1260, Aroclor 1242 proved the most toxic, causing 100% mortality at 400 ppm in the diet and 40% mortality at 200 ppm in 4 weeks; Aroclor 1254 caused 60% mortality at 400 ppm and 20% mortality at 200 ppm; Aroclor 1260 caused no mortality even at 400 ppm (65). Together these studies show that chickens are more sensitive than any of the species listed in Table III.6.1; also in contrast to these species, chickens are more sensitive to the mixtures with lower chlorination. Marked variations in the toxicity of PCB mixtures to chickens have been noted (2, 136: see next section).

The toxicity of PCBs has been studied in several other species of birds. In Japanese quail, 2000 ppm of Aroclor 1254 in the diet caused 100% mortality between 6 and 55 days (139). In Bengalese finches, a dose rate of about 250 mg/kg/day of Aroclor 1254 was needed to cause 50% mortality in 56 days (140), but the dose-response curve was very shallow and some birds died even at the lowest treatment level (about 6 mg/kg/day). Some mortality was caused among subadult pheasants by doses of Aroclor 1254 as low as 10 mg per day (about 15 mg/kg/day) (141). Cormorants treated with Clophen A60 at 200-400 ppm in the diet died after 55-124 days (142).

Residues of PCBs in the brains of experimentally killed birds were in the range 350-760 ppm in blackbirds (130), 300-400 ppm in pheasants (141) and 75-180 ppm in cormorants (142). Brain residues in these ranges are believed to be indicative of death by PCB poisoning (130). Residues in the livers of poisoned birds were in the range 200-300 ppm in cormorants (142) and 70-700 ppm in Bengalese finches (141), but much higher in pheasants (142).

No investigations appear to have been reported of the toxicity of Aroclor 1016 to birds.

III.6.2 Chick edema disease and the role of PCDFs in PCB toxicity.

The most characteristic signs of toxicity in chickens experimentally poisoned with PCBs are subcutaneous edema, hydropericardium, and in some cases enlargement of the liver, liver necrosis, reduction in the size of the spleen, and kidney damage (Table III.6.2; 65, 129, 132-139). Hydropericardium has also been recorded in some, but not all, treated individuals of Bengalese finches (140), Japanese quail (139) and pheasants (141), but not cormorants (142). Atrophy of the spleen and liver changes were prominent in poisoned pheasants (141). Hemorrhaging in breast muscle and other tissues has also been reported (65, 138).

Edema and hydropericardium are characteristic signs of "chick edema disease", a syndrome which periodically affects flocks of domestic chickens. The cause of "chick edema disease" has been traced to toxic fat included in commercial feeds, and the usual toxic agent has been identified as polychlorinated dibenzodioxins (PCDDs) (143, 67). However, outbreaks of chick edema disease in Japan in 1968 and in the United States in 1971 were traced to contamination of chicken feed with PCBs (144, 65). At least in the latter case, the concentration of PCBs in the feed seemed insufficient to account for its toxicity (65; 2, pp. 327-328). This prompted the search for another toxic contaminant.

Vos and Koeman (137) found that under identical conditions Clophen A60 and Phenoclor DP-6 were much more toxic to chicks than Aroclor 1260, and that most of the classic signs of chick edema disease were caused by the first two mixtures (Table III.6.3). On fractionating the mixtures most of the toxicity was traced to the most polar fractions of Clophen and Phenoclor and compounds tentatively identified as PCDFs (4-Cl and 5-Cl) were identified in them. Subsequently PCDFs have also been identified in the PCBs which caused the Japanese incident in 1968 (34).

TABLE III.6.3 (from ref. 137)

MORTALITY, MEAN SURVIVAL TIME AND PATHOLOGIC OBSERVATIONS OF CHICKS FED 400 ppm PCB FOR 60 DAYS AND OF CONTROL BIRDS

Groups	N	Number of deaths	Mean survival[a] time (days)	Number of birds with edema			Number of birds with liver necrosis
				Hydropericardium[b]	Abdominal	Subcutaneous	
I (Phenoclor)	24	24	24.3 (12–58)	18	8	5	9
II (Clophen)	22	22	20.5 (13–29)	20	9	7	9
III (Aroclor)	20	3	—	3	0	0	0
IV (Control)	20	0	—	0	0	0	0

[a] The range of values is shown in parentheses.
[b] Positive hydropericardium response was considered to be over 0.2 ml pericardial fluid in chicks 20 days of age and 0.4 ml in chicks 60 days of age, with interpolation for intermediate ages.

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In recent direct studies, 2,3,7,8-tetrachlorodibenzofuran (TCDF) proved exceptionally toxic to chicks (30, 71). Birds which were treated with 5 μg/kg/day TCDF died in an average of 11.5 days. Chicks which were treated with 1 μg/kg/day started to die on the 19th day, and were in very poor condition when the experiment was terminated. The most striking gross pathologic change observed was the accumulation of clear fluid, as evidenced by marked subcutaneous edema, ascites, and hydropericardium, with the severity of the fluid accumulation greatest at the 5 μg/kg dose. The hearts of these birds also appeared enlarged and flabby (rounded). The second striking change was a marked reduction in size of the thymus and spleen. Histologically, size reduction could be accounted for by the marked depletion of lymphocytic cell types in the spleen and cortex of the thymus. Similar pathologic changes were found to occur at the 1 μg/kg dose, albeit to a milder extent. There were no significant effects observed on liver weight; mild liver pathology was found to occur only at the 5 μg/kg dose. Total serum protein levels were reduced with a marked reduction in serum albumin (71).

Although this experiment and that of Vos and Koeman (137) indicate that PCDFs are major causes of chick edema disease, they do not exclude the possibility that some PCBs do so also. For example, Aroclor 1260 caused some deaths and hydropericardium in Vos and Koeman's experiment (Table III.6.3), although the intake of PCDFs by these chicks would have been only about 50 ng/kg/day (400 ppm Aroclor x 0.8 ppm PCDF x 160 g/kg/day food intake; refs. 32, 137). Mortality and hydropericardium have been recorded in other species treated at even lower dose rates of Aroclor 1254 (65, 140, 151). In pheasants, marked atrophy of the spleen, liver enlargement and degeneration, and one case of hydropericardium were induced by daily doses as low as 15 mg/kg/day of Aroclor 1254, an intake which is unlikely to have represented more than 25 ng/kg/day of PCDFs (141, 32).

In another recent study, chicks were dosed with five symmetricel isomers of hexachlorobiphenyl (30, 70). Although four of these had little effect except on the liver (and slight effects on the thymus) at doses of 400 ppm, 3,4,5,3',4',5'-hexachlorobiphenyl proved exceptionally toxic, causing 100% mortality even at 3 ppm in the diet and producing the classic symptoms of chick edema disease at that level (Tables III.6.4, III.6.5). This isomer apparently is not present in commercial PCB mixtures, although structurally similar isomers are present in small quantities (Section II.5.4 above). It is not yet clear whether the effects of this isomer are due to its structural similarity to the most toxic CDFs and CDDs, or whether CDFs may be produced from it by metabolism.

It is therefore impossible with existing information to disentangle the roles of PCBs and PCDFs in chick edema disease. Although PCDFs seem to play a major role in the toxicity of Clophen A60 and Phenoclor DP-6 to chickens (137), the same symptoms are produced by at least one specific PCB isomer. Further, some of the symptoms of chick edema disease are produced by Aroclor mixtures which contain extremely small quantities of PCDFs, and most of the effects on the liver appear to be attributable to PCBs themselves (129). For further discussion of the environmental significance of PCDFs see below in Section III.16.

Chick edema disease has also been induced experimentally with polychlorinated naphthalenes (67) but this effect also is probably attributable at least in part to PCDFs (see Section III.16).

III.6.3. Porphyria and induction of ALA-synthetase

Vos and Koeman (137) found that experimental poisoning of chicks with PCBs was accompanied by increased fecal excretion of coproporphyrin and

TABLE III.6.4 (from ref. 70)

Summary of the biological effects of hexachlorobiphenyl isomers in chicks

Chemical	Dose level (ppm)	Mortality	Body weight gain (% of control)	Liver effect	Porphyrin Accumulation	Thymus effect
3,4,5,3',4',5'-HCB	3	10/10	-	+++	-	+++
	10	10/10	-	++	+++	+++
2,3,4,2',3',4'-HCB	400	0/8	68	++	-	+
2,4,5,2',4',5'-HCB	400	0/10	66	++	-	+
2,3,6,2',3',6'-HCB	400	1/10	80	++	-	+
2,4,6,2',4',6'-HCB	400	0/10	83	+++	-	+

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TABLE III.6.5 (ITC [illegible] 70, 30)

Summary of major pathological changes in chicks given different hexachlorobiphenyl isomers for 21 days

Chemical	Dose level (ppm)	Liver	Thymus	Spleen	Fluid Accumulation
3,4,5,3',4',5'-HCB	3	Marked; fatty metamorphosis, focal necrosis.	Marked; involution.	Marked; depletion of lymphocytes.	Subcutaneous edema, ascites, hydropericardium.
2,3,4,2',3',4'-HCB	400	Moderate; hyalinization, single-cell necrosis.	Slight; Involution.	NS[a]	Epicardial edema.
2,4,5,2',4',5'-HCB	400	Moderate; single-cell necrosis.	Slight; involution.	NS[a]	NS[a]
2,3,6,2',3',6'-HCB	400	Moderate; fatty metamorphosis, focal necrosis, giant cell formation.	Slight; involution.	NS[a]	NS[a]
2,4,6,2',4',6'-HCB	400	Marked; fatty metamorphosis, focal necrosis, giant cell formation, marked focal dilatation of sinusoids.	Slight; involution.	NS[a]	NS[a]
2,3,7,8 -TCDF	5 μg/kg	Mild; passive congestion	Marked; involution	Marked; depletion of lymphocytes	Subcutaneous edema, ascites, hydropericardium

[a]NS = not significant.

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protoporphyrin and fluorescence of tissues. Chicks which died during the experiment had a much higher incidence of fluorescence in liver, bile, and small intestine than did those that survived; fluorescence in bone was prominent in both groups. The effects were similar in chicks treated with Aroclor 1260, Clophen A60 and Phenoclor DP-6, suggesting that the porphyria is an effect of PCBs themselves rather than PCDFs (129).

In a subsequent study with Japanese quail, Vos et al. (145) found that treatment with Aroclor 1260 at doses of 100 mg/kg/day for 7 days or greater induced porphyrin fluorescence in the liver. Mitochondrial activity of δ-aminolevulinic acid (ALA)-synthetase was greatly increased. Together with the doubling of mitochondrial protein, liver enlargement, and pyroninophilic staining of liver nucleoli, this suggested an increased protein synthesis (145). Further testing at lower doses showed a significant induction of ALA-synthetase at 1 mg/kg and a marked but non-significant increase even at 0.1 mg/kg (Table III.6.6). A significant increase in ALA-synthetase was associated with PCB residues in the liver as low as 1 ppm (Figure III.6.1). This is one of the lowest levels of PCB exposure associated with a measurable effect in an experimental animal.

Testing five individual isomers of hexachlorobiphenyl, Goldstein et al. (146, 147) found that all five stimulated ALA-synthetase, but that there were marked differences in their porphyrinogenic effects (Tables III.6.5, III.6.7). The 3,4,5,3',4',5'- isomer had by far the greatest effect; the 2,4,5,2',4',5'- and 2,3,4,2',3',4'- isomers also had marked effects, but the other two isomers had no significant effects. 2,3,7,8-TCDF had no effect either on ALA-synthetase or porphyrin accumulation (Table III.6.7). The lack of effect of 2,3,7,8-TCDF contrasts strongly with that of the structurally similar 2,3,7,8-TCDD, which is a potent inducer of ALA-synthetase in chicks (148). Furthermore, the

TABLE III.6.6
(from ref. 145)

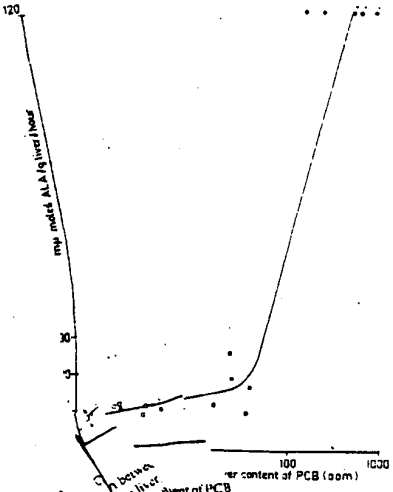
FORMATION OF δ-AMINOLEVULINIC ACID BY LIVER MITOCHONDRIA, CYTOCHROME P-450, PROTEIN CONTENT OF THE MITOCHONDRIAL FRACTION, LIVER WEIGHTS, LIVER RESIDUES, AND TISSUE FLUORESCENCE IN FEMALE JAPANESE QUAIL ORALLY DOSED WITH PCB FOR 7 DAYS[a]

Aroclor 1260 (mg/kg body weight)	ALA formed (nμmoles ALA/g liver/hr)	Cytochrome P-450 (nμmoles/ g liver)	Protein content mitochondrial fraction (mg/g liver)	Liver (g)	Liver (g/100 g body weight)	PCB Content liver (ppm)	Tissue fluorescence incidence	
							Macroscopic	Microscopic (liver)
0	6.46 ± 1.65	< 9.1	19.1 ± 5.3	4.43 ± 0.51	3.13 ± 0.13	0.15[c]	0/5	0/5
0.1	8.76 ± 4.06	< 6.9	17.7 ± 1.8	4.31 ± 0.62	3.09 ± 0.51	0.54 ± 0.27	0/5	0/5
1	10.50 ± 1.21[b]	< 4.3	20.9 ± 3.7	4.22 ± 0.76	2.76 ± 0.35	1.41 ± 0.67	0/5	0/5
10	17.3 ± 6.4[b]	14.0	17.8 ± 2.5	5.09 ± 1.19	3.52 ± 0.65	27.0 ± 9.4	0/5	0/5
100	119.9[c]	19.7[c]	29.1[c]	4.73 ± 0.82	3.89 ± 0.63	478 ± 294	3/5	2/5

[a] Mean values ± SD, 5 birds per group.
[b] Significantly different from controls, $P < 0.01$.
[c] Pooled samples.

MONS 064301

Fig. III
(from ref.



...(mo... between ... g liver ... ent of PCB
mg/kg = b... ...wing ... animals: ... m) and the mitochondrial ALA synthetase
contrgroup ... determin... ...ultraviol... ng/kg: □, 1 mg/kg; ■, 10/mg/kg; ●, 100
tion of the ALA ... thetase ac... ...ontent, a... ...t. Liver samples were pooled in the
... ...e 100 mg/kg group for the determina-

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EFFECT OF 400 PPM PURIFIED HEXACHLOROBIPHENYL ISOMERS AND TCDF ON CHICK LIVERS[a]

Treatment	ALAS nmol/g/hr	Incidence of Porphyria[c] (> 10 µg/g)	Liver Porphyrin Levels (µg/g) (Range)
Control	55 ± 2	0/10	0.4 - 1.4
2,3,6,2',3',6'-HCB	261 ± 15[b]	0/10[d]	0.5 - 1.4
2,4,6,2',4',6'-HCB	303 ± 15[b]	0/10[d]	0.7 - 2.9
2,4,5,2',4',5'-HCB	309 ± 13[b]	3/9 [d]	1.5 - 82
2,3,4,2',3',4'-HCB	250 ± 28[b]	2/8 [d]	1.2 - 81
3,4,5,3',4',5'-HCB	DEAD	1/7 [d]	2.4 - 5,000
Corn Oil	74 ± 2	0/33	0.4 - 1.2
1 µg/kg/day TCDF x 21 days	63 ±20	0/5	0.4 - 2.9
5 µg/kg/day TCDF x 21 days	DEAD	0/5	0.3 - 0.7

[a] Treatment described in Fig. 1.

[b] Significantly greater than controls, $P < 0.05$ (Duncan's Multiple-Range test).

[c] Proportion of animals whose liver porphyrin levels exceed 10µg/g. The upper 99% tolerance levels for controls at $P < 0.01$ is 2.3 µg/g.

[d] Uroporphyrin detectable in all samples after thin-layer chromatography.

Table III.6.7 (from ref. 147)

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strong contrast between 3,4,5,3',4',5'-hexachlorobiphenyl and 2,3,7,8-TCDF in their effects on ALA-synthetase and porphyrogenesis (Table III.6.7) show that contamination with the latter compound cannot account for the extreme potency of the former in producing chick edema disease (Table III.6.4).

Only hexa-chlorobiphenyls and Aroclor 1260 appear to have been tested for these effects in birds, but since lower chlorobiphenyls and mixtures are also active in rats (see below) there is no reason to assume that the effects in birds would be limited to the more highly chlorinated compounds.

III.6.4 Effects on reproduction

III.6.4.1 Effects in chickens

Numerous studies have been carried out of the effects of PCBs on reproduction in chickens, because of their apparent sensitivity and because of several commercially damaging incidents (65, 144).

In an experiment reported by Scott et al. (149, 150), Aroclor 1248 was fed to White Leghorn chickens at dietary levels of 0.5, 1, 10 and 20 ppm. Egg production was significantly reduced in the 10 and 20 ppm groups after 8 weeks. There was no significant reduction in the breaking strength of eggs at any dose level, but hatchability was severely reduced in the groups fed 10 and 20 ppm. Hatchability was reduced to 72% of normal when the PCB level in the eggs reached 2.2 ppm (wet weight), to about 56% at 3 ppm, and almost to zero at 4.5 ppm.

In another study, White Leghorn and broiler hens were fed diets containing 20 and 50 ppm Aroclor 1242 for six weeks. Even at 20 ppm hatchability was reduced to half that in controls by the second week of feeding and almost to zero in the fourth and fifth weeks. Broiler hens were slightly more resistant than White Leghorns. Most of the embryonic mortality took

place in the middle and late stages of development. No hydropericardium was observed in the affected embryos, but abnormal anatomy of the heart was observed, involving ridges on the exterior of the right ventricle (151).*

In another study involving several dose levels of Aroclor 1242, there was no effect on egg production, egg weight, or eggshell thickness at any dose level. Hatchability was reduced in the group fed 10 ppm for six weeks, and reduced markedly in the groups fed 20, 40 or 80 ppm. Significant reductions in hatching success were noted when PCB residues in the yolk exceeded 2.4 ppm (about 1.0 ppm in the whole egg). Hatchability recovered almost to normal after a 6-week period on clean food in which the yolk residues fell below 1.8 ppm (152).

In another study involving feeding Aroclor 1254 at dietary levels of 5 and 50 ppm, egg production and fertility were reduced at both exposure levels; hatchability of fertile eggs was effected only in the 50 ppm group (153).

In another study in which Aroclor 1254 was included in drinking water at 50 ppm (corresponding to an intake of about 6.3 mg/kg body wt/day), fertility was unaffected but hatchability dropped to zero by the third week. Hatchability started to increase after nine weeks on uncontaminated diets, but did not return to normal until about the 17th week, when yolk residues had declined to about 10 ppm (Figure III.6.2). Deformities of the legs, toes, and neck were observed in many chicks hatching from eggs with yolk levels 10-15 ppm or more (154, 155). Other developmental defects were observed in the few chicks surviving these concentrations (154).** Teratogenic effects were also observed when 5 mg Aroclor 1242 was injected into the yolk sac of fertile eggs (156).

In a comparative study, White Leghorn chickens were treated with Aroclors 1016, 1232, 1242, 1248 and 1254 for eight weeks at dietary levels

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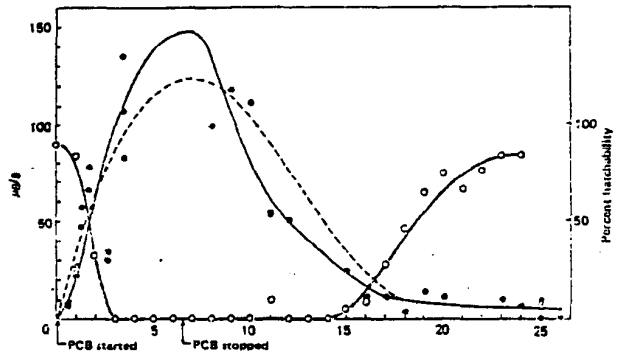
* Abnormalities of the epicardium (fenestration and fibrous degeneration) were reported in another study involving feeding of Aroclor 1254 (400).

** Toe deformities were also seen in birds of the second (F_2) generation, suggesting a mutagenic effect (155, p. 204).

Figure III.6.2
(from ref. 155)



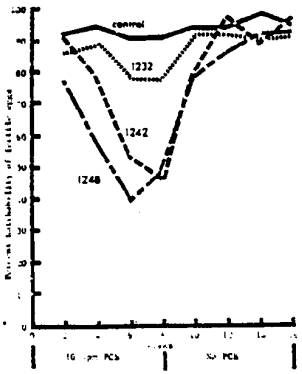
PCB levels in egg yolk, determined by TLC, and hatchability of experimental eggs. • = PCB level; ———— = hand-interpolated curve; – – – – = least-squares fit to a third-order regression, ○ = hatchability.

of 5, 10 and 20 ppm. None of the mixtures affected egg production, egg weight, eggshell thickness or food consumption. Hatchability of fertile eggs was severely reduced by Aroclors 1232, 1242 and 1248 at the 10 or 20 ppm levels (Fig. III.6.3), but there were no significant effects induced at 5 ppm, nor by Aroclors 1016 or 1254 at any level. The growth rate of the surviving chicks was reduced by maternal exposure to all 5 mixtures (chicks were exposed only via the egg); this reduction was most marked for Aroclors 1242 and 1248. Embryonic death was most pronounced in two periods of development: on days 3-10 and after the eggs were pipped (Table III.6.8). Abnormal embryos were found in eggs treated with Aroclors 1242 and 1248 (Table III.6.8): abnormalities included edema, unabsorbed yolk, small bodies, external hemorrhage, and leg malformations (157). In an earlier study with 20 ppm Aroclors 1242 and 1248 as many as 33-50% of the dead embryos showed these abnormalities (158). In this earlier study Aroclors 1221 and 1268 appeared to have no effects on chicken reproduction at levels of 20 ppm (158).

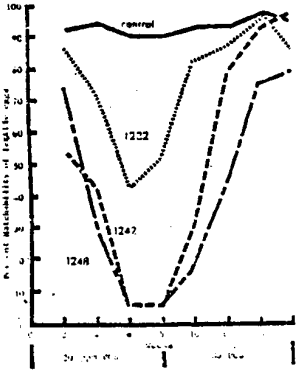
In another comparative study with White Leghorn chickens no effects were found with Aroclor 1260 at any dose level up to 100 ppm; Aroclor 1254 affected egg production and hatchability at 100 ppm, and chick viability was reduced at 10 ppm; Aroclor 1242 reduced egg production at 100 ppm, chick viability at 10 ppm, and hatchability at 8 ppm, but not at 4 ppm (68). Eggshell thickness is said to have been reduced by Aroclor 1242 at 10 and 100 ppm, but no details were given and this result conflicts with that obtained by other experimenters.

Finally, in the East Coast Terminal incident in which commercial chicken feed was contaminated with Aroclor 1248 (65), hatchability of eggs from chickens ingesting the feed dropped to about 2% (2). In affected

Figure III.6.3
(from ref. 157)



Effect of 10 p.p.m. PCB feeding and subsequent PCB withdrawal on hatchability of fertile eggs in a 16-week period.



Effect of 20 p.p.m. PCB feeding and subsequent PCB withdrawal on hatchability of fertile eggs in a 16-week period.

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TABLE III.6.8
(from ref. 157)

—*Fertility, hatchability, abnormal embryos and peak of embryonic death during feeding of Aroclors 1242 and 1248 in the maternal diet*

PCB	PCB level	Fertile eggs	Hatch	Abnormal embryos as % of		Embryonic death % on incubation day				Pipped eggs
Aroclor	p.p.m.	Number	% of fertile eggs	Fertile eggs	Dead embryos	1–2	3–10	11–19	20–21	% of day 21 dead
None	0	163	91	2	20	53	7	26	14	9
1242	5	106	76	8	35	54	15	0	31	33
1248	5	109	87	2	14	36	21	7	36	40
1242	10	103	47	9	26	12	47	9	32	43
1248	10	114	69	5	17	11	40	0	49	29
1242	20	194	28	12	17	4	38	13	45	29
1248	20	204	22	13	16	9	33	9	45	31

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samples of eggs, fresh yolk prior to incubation contained about 0.29 ppm, yolk of fertile eggs contained 0.97 ppm and 1-day-old dead embryos contained 0.35 ppm (2). Comparison with the levels required to reduce hatchability in other experiments (149, 150) indicates that the material which leaked from the heat exchanger was considerably more toxic than fresh Aroclor 1248 (2: see Section II.8.4 above).

To summarize these studies with chickens, marked effects on reproductive success have been noted at dietary levels as low as 8-10 ppm, corresponding to residues of only 1-2 ppm in whole eggs. Aroclors 1242 and 1248 are much more potent than higher or lower chlorinated mixtures, but Aroclor 1016 was much less active than Aroclor 1242 in one test. The principal cause of reproductive failure is embryonic mortality, and abnormal embryos and teratogenic effects have been reported in several experiments.

The possible role of PCDFs in embryonic mortality is not clear. PCDFs appear to be embryotoxic in chickens (129) and the high toxicity of the PCBs in the East Coast Terminal incident suggests the presence of a toxic contaminant (2). However, it is difficult to attribute the greater toxicity of Aroclor 1242 than Aroclor 1016 (157) solely to the presence of PCDFs in the former mixture, because Aroclors 1254 and 1260 (which also contain PCDFs - 32) appear much less embryotoxic (157, 68). It seems more plausible to attribute the high toxicity of Aroclors 1242 and 1248 in chicken embryos to the presence of a specific tetrachlorobiphenyl which is less well represented in Aroclors 1016 and 1254. Ax and Hansen (399) report that 2,4,5,3',4'- pentaCB is very toxic to chick embryos, but this isomer is not present in Aroclor 1242.

III.6.4.2 Effects in Pheasants

Dahlgren et al (141, 159) dosed female pheasants with Aroclor 1254 by capsule at rates of 12.5 or 50 mg weekly (approximately 2.5 and 10 mg/kg/day, equivalent to about 50 and 200 ppm in the diet). Egg production was

significantly reduced in all treated groups, and there was a significant increase in the number of eggs that were pipped but not hatched. Although egg fertility and hatchability were not significantly reduced, survival of chicks was reduced in all groups and the overall reproductive success was significantly reduced. No effects were found on eggshell thickness (141).

III.6.4.3 Effects on Mallards

In a two-year study with Mallards, Aroclor 1254 at 25 ppm in the diet had no significant effects on any aspect of reproductive success except for a reduction in the number of eggs laid, which took place only in one of four pens and was believed to be unrelated to treatment (131) (Table III. 6.9). Eggshell thickness was unaffected by Aroclor 1254, although in a parallel experiment DDE caused significant eggshell thinning (Table III.6.10). Residue levels in eggs (33 ppm wet weight) were much higher than those associated with embryonic mortality in chickens.

Similar results were reported in another experiment in which Mallards were fed 40 ppm Aroclor 1254, 40 ppm DDE, and the two compounds together (160). Unlike DDE, Aroclor 1254 did not cause significant eggshell thinning, and it caused only a slight and non-significant increase in the degree of eggshell-thinning induced by DDE (Table III.6.11). However, egg production was markedly reduced in the Mallards fed PCBs and DDE simultaneously, and there was a significant increase in egg-eating by the birds on combined dosage (160).

III.6.4.4 Effects in Bobwhite Quail

Reproduction in bobwhite quail was not measurably affected by dietary exposure to either Aroclor 1254 or DDE, administered singly or in combination (131) (Table III.6.12).

TABLE III.6.9
(ref. 131)

REPRODUCTIVE SUCCESS AMONG PENNED MALLARDS MAINTAINED FOR TWO SEASONS[1] ON FEEDS CONTAINING AROCLOR 1254 OR DDE

	season	chemical added to feed (ppm)		
		control	Aroclor 1254 (25)	DDE (10)
Pens of birds (5 ♀♀, 2 ♂♂ each)	1969	4	4	4
	1970	4	4	4
Hen-seasons (8 weeks each)	1969	20	20	19.1
	1970	20	19	18
Total eggs laid	1969	590	433	623
	1970	620	468	622
Eggs laid per hen-seasons	1969	29.5	21.7	32.3
	1970	31.5	24.6	33.1
Eggs cracked (%)	1969	4	6	10[2]
	1970	9	9	28[2]
Eggs embryonated of eggs set (%)	1969	86	94	80
	1970	88	90	63
Embryos alive at 3 weeks (%)	1969	98	96	94
	1970	92	92	88
Normal hatchlings of 3-week embryos (%)	1969	70	63	47[2]
	1970	59	64	53
Normal hatchlings alive at 14 days (%)	1969	94	91	97
	1970	93	96	92
14-day ducklings of embryonated eggs (%)	1969	64	55	42[2]
	1970	53	57	48[2]
14-day ducklings per hen-seasons	1969	13.0	8.8	7.8[2]
	1970	11.6	10.4	6.7[2]

[1] Dosage started December 19, 1968; eggs collected from March 12 through May 6, 1969, and from March 18 through May 12, 1970

[2] Differences from controls statistically significant ($P < 0.05$).

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TABLE III.6.10
(ref. 131)

MEAN THICKNESS OF EGGSHELLS OF PENNED MALLARDS MAINTAINED THROUGH TWO SEASONS ON FEED CONTAINING AROCLOR 1254 OR DDE

		chemical added to feed (ppm)		
	season	none	Aroclor (25)	DDE (10)
No. of eggs	1969	28	27	27
	1970	32	17	26
Shell thickness (mm)	1969	0.375	0.360	0.339[1]
	1970	0.365	0.308	0.328[1]

[1] Differences from controls statistically significant ($P < 0.05$).

MONS 064313

TABLE III.6.11
(from ref. 160)

Mean eggshell thickness indices for 1-day egg collections from capes of mallards fed four experimental diets.

Treatment	Single 1-day egg collections						Largest 1-day collections during 3-day periods[a]			
	Day 30		Day 32		Day 50		Days 30–32		Days 50–52	
	No. eggs	Mean index[b]	No. eggs	Mean index	No. eggs	Mean index	No. eggs	Mean index	No. eggs	Mean index
Control	7	0.212 A	5	0.214 A	5	0.213 A	9	0.212 A	5	0.217 A
PCB	4	0.193 AB	4	0.211 AB	8	0.216 AB	5	0.201 AB	9	0.216 AB
DDE	6	0.177 BC	5	0.150 C	7	0.170 C	6	0.177 C	5	0.172 C
DDE + PCB	8	0.164 C	8	0.170 C	8	0.166 C	9	0.167 C	8	0.166 C
F-values[c]		7.73		12.00		29.00		18.50		32.00

[a] Values for each treatment represent combined data for the single largest 1-day egg collection during the 3-day period from each of two replicate cages.
[b] Within a column, means followed by the same letter are not significantly different ($P < 0.05$).
[c] All F-values are significant ($P < 0.005$).

MONS 064314

III.6.4.5 Effects in Ring Doves

Peakall et al. (161) studied reproduction in ring doves exposed to 10 ppm Aroclor 1254 for two generations. Although there were no effects in the first generation, there was high embryonic mortality in the second generation (Table III.6.13). Cytogenetic studies of treated embryos showed a number of chromosomal aberrations (primarily chromatid, some isochromatid, and one rearrangement). Thirteen of 17 treated embryos had aberration rates exceeding the mean rate in controls (161). Eggshell thickness was not affected (162). In a subsequent study it was found that embryonic mortality was due in part to abnormal incubation behavior by the PCB-treated parents (163). Eggs incubated artificially, or by untreated parents, had a higher hatching success. However, significant embryonic mortality was observed even in artificially incubated eggs, and this was attributed primarily to the chromosomal effects (163).

III.6.4.6 Effects in American Kestrels

Because residue levels of PCBs are often very high in predatory birds (1, 2, 6) special interest attaches to an experiment in which the effects of dietary PCBs were studied in captive American Kestrels (164). Groups of breeding Kestrels were fed diets containing 10 ppm Aroclor 1254 (wet weight of diet), 3 ppm DDE, and a combination of the two chemicals. Eggshell-thickness was slightly increased in the PCB-treated birds (statistically significant in one experiment, but not in others), markedly decreased in the DDE-treated birds, and still further decreased in the birds treated with DDE and PCBs (Table III.6.14). The difference between the birds treated with DDE and those treated with both chemicals was statistically significant. All the eggs from birds treated with both chemicals were broken during incubation (Table III.6.14). When eggs were artifically incubated, embryonic

TABLE III.6.12
(from ref. 131)

REPRODUCTIVE SUCCESS AMONG PENNED BOBWHITE FED DIETS CONTAINING AROCLOR 1254, DDE, OR A MIXTURE OF BOTH CHEMICALS[1]

	chemical added to feed (ppm)			
	control	Aroclor (50)	DDE (30)	Aroclor 1254 + DDE (25) + (15)
Pens of birds (3 ♂♂, 2 ♀♀ each)	6	6	6	5
Hen-seasons (8 weeks each)	16.6	16.4	13.3	14.9
Total eggs laid	347	368	415	386
Eggs laid per hen-season	10.9	22.4	27.1	26.0
Eggs cracked (%)	10	7	5	7
Eggs embryonated of eggs set (%)	88	90	93	93
Embryos alive at 3 weeks (%)	97	97	98	97
Normal hatchlings of 3-week embryos (%)	96	91	96	92
Normal hatchlings alive after 14 days (%)	78	72	70	71
14-day chicks of embryonated eggs (%)	73	64	66	65
14-day chicks per hen-season	13.5	11.5	15.1	13.3

[1] Dosage started April 5, 1968; eggs collected from April 27 through June 21, 1968.
None of the above differences was significant at the 95 percent level of confidence.

MEAN THICKNESS OF EGGSHELLS OF PENNED BOBWHITE MAINTAINED THROUGH ONE SEASON ON FEED CONTAINING AROCLOR 1254, DDE, OR A MIXTURE OF BOTH CHEMICALS

	chemical added to feed (ppm)			
	none	Aroclor (50)	DDE (30)	Aroclor 1254 + DDE (25) + (15)
No. of eggs	14	17	20	18
Shell thickness (mm)	0.207	0.208	0.205	0.215

None of the above differences was significant at the 95 percent level of confidence.

MONS 064316

TABLE III.6.13
(from ref. 172)

Breeding success of Ring Doves fed 10 ppm PCBs over two generations.

	No. of pairs	No. of eggs laid	No. of eggs hatched	No. of young fledged
Pre-experiment	6	24	22	22
1st generation PCBs	6	24	24	24
2nd generation PCBs	6	20*	4	2**
2nd generation Control	6	24	22	22

* One pair failed to produce any eggs during the period of the experiment.

** Fledging losses consisted of one young found outside the nest at one day of age and one deformed bird killed at the age of three weeks.

Table III.6.14 (from ref. 164)

Kestrel Eggshell Thinning in Response to Chronic Dietary p,p'-DDE and/or Aroclor 1254 Based on (A) Ratcliffe's Index*, (B) Direct Measurement of Shell With Membranes(mm), (C) Incidence of Egg Breakage**.

	Control	3 ppm DDE	10 ppm 1254	3 ppm DDE + 10 ppm 1254
		(A)		
# of Eggs	15	17	18	12
$\bar{x}$ ± s.d.	0.920 ± .050	0.799 ± .054	0.977 ± .080	0.770 ± .045
Standard Error	0.010	0.010	0.017	0.010
% Difference from Control	0.00	-13.16	+6.19	-16.31
Statistical Significance tt	—	< .0005	< .005	< .0005
		(B)		
# of Eggs	15	18	14	15
$\bar{x}$ ±	0.171 ± .013	0.145 ± .009	0.176 ± .015	0.139 ± .008
Standard Error	0.003	0.002	0.004	0.002
% Difference from Control	0.00	-15.21	+2.92	-18.72
Statistical Significance tt	—	< .0005	< .025	< .0005
		(C)		
	0(3)	0(5)	0t(5)	100(5)

* Oven dry shell weight (mg/length x breadth of egg (mm)).
** Percent of total number of pairs; number of pairs in parentheses.
t One pair exhibited breakage but was attributable to other causes.
tt Probability of a larger Student's t value when compared with controls using a one-tailed t-test (Steel and Torrie, 1960; Table A. 3).

MDNS 064318

mortality was increased in all treated groups but was greatest (100%) in the eggs from birds treated with both chemicals (164). Thus, while 10 ppm Aroclor 1254 appeared to cause some embryonic mortality, it also significantly potentiated the effects of DDE in causing eggshell thinning, egg breakage, and embryonic mortality. PCB residues in the eggs of the jointly-dosed groups averaged 184 ppm (dry weight), i.e., about 30 ppm, wet weight.

III.6.4.3 Effects in Japanese quail

Japanese quail appear relatively resistant to reproductive effects of PCBs and have been tested only at high dietary levels. Dietary exposure to 312 ppm Aroclor 1242, 78 ppm Aroclor 1254, and 62.5 ppm Aroclor 1260 caused a significant reduction in egg production and significant reductions in eggshell thickness (by 4%, 12% and 10% respectively). Egg weights were also reduced in birds fed Aroclor 1260 (165). In another experiment 100 ppm Aroclor 1242 caused a reduction in egg production, but 100 ppm Aroclor 1254 did not. Aroclor 1242 increased the number of broken eggs (166). Dietary exposure to 50 ppm Aroclor 1254 caused only a slight and non-significant reduction (4.5%) in eggshell thickness (167).

III.6.4.4 Summary of effects on reproduction in birds

There are marked differences between species in sensitivity to PCBs. Chickens are very sensitive, particularly to Aroclors 1242 and 1248; their reproduction is markedly affected by dietary levels of 8-10 ppm, corresponding to residues of 1-4 ppm in eggs. Ring doves were seriously affected by dietary exposure to 10 ppm over two generations, and American Kestrels by exposure to 10 ppm in combination with DDE. In contrast, mallards, bobwhite quail and Japanese quail are much less affected by PCBs. There is little evidence of significant effects on eggshell thickness, except in kestrels in

combination with DDE. Indications of synergistic effects between PCBs and DDE on reproduction in kestrels and mallards may have important ecological implications, as may the second-generation effects in ring doves.

III.6.5 Liver and microsomal enzymes

The liver has been the primary organ used in delineating effects of PCBs on birds. This concentration of interest can be attributed to the high liver PCB levels found, and to the responsibility of the hepatic systems for metabolizing foreign compounds. Increases in liver weight and size are commonly noted (157, 168). Increase in amount of liver lipids present and a decrease in the storage of liver vitamin A have been frequently reported (158, 166). The liver vitamin A storage reduction has not evidenced symptoms of vitamin A deficiency. However, it was speculated that birds intaking marginal dietary levels of vitamin A, coupled with the liver vitamin A storage reduction, might be susceptible to avitaminosis.

Elevations of the liver cytoplasmic RNA content in PCB-treated birds have been related and correlated with increases in hepatic enzyme activity. Lower cytophotometric readings of percent transmission in experimental livers was determined to be a consequence of increased cytoplasmic RNA (169). Bailey and Bunyan (170) noted increased cytochromeP-450 levels which are indicative of microsomal induction in the livers of birds given PCBs. These elevated levels existed for six months after the withdrawal of dietary PCBs indicating that extremely low PCB residues can cause significant increases in microsomal enzymatic activity.

Several hepatic enzymes have been reported to be induced by PCBs. Evaluation of the liver enzyme controlling pentoberbital metabolism (166) revealed that PCBs exhibit a diphasic effect on pentobarbital induced sleeping times. The PCBs initially inhibit the enzymes metabolizing penta-

barbital. Subsequently, these same enzymes are induced, by the PCB stimulation, to greater activity yielding more rapid than normal metabolism of pentobarbital.

Estradiol degradation is another function which PCBs have been found to alter. Lincar and Peakall (169) reported a dose-dependent breakdown of estradiol to a more polar metabolite. Nowicki and Norman (171) reported increased *in vitro* hepatic metabolism of two additional natural steroids: testosterone and 4-androstene-3,17-dione. These two were reduced to polar metabolites as was estradiol. The biological effects of this increase in metabolism of sex hormones to polar forms is relatively unknown but it may be a significant contribution to reproductive problems and failures that have been reported.

A study of hepatically-directed plasma enzyme activity was conducted by Dieter (172). Sublethal doses in quail produced elevation in enzyme activity and reductions in hematocrit and hemoglobin concentrations. The plasma enzymes increased were: (1) cholinesterase by 80%; (2) fructosediphosphate aldolase by 5 to 6 fold; (3) creatine kinase, 4 fold; (4) lactate dehydrogenase, 9 fold; and (5) aspartate aminotransferase by 75%. ChE, LDH, and AAT elevations were dose-related enzyme releases which could be used to quantitatively establish PCB exposure. Qualitative exposure assays were judged possible due to differentiation of enzyme activity in birds exposed to DDE, malathion, mercuric chloride and Aroclor 1254.

Goldstein *et al.* (147) studied the effects of five isomeric hexachlorobiphenyls and 2,3,7,8-TCDF on liver enzymes in the chicken in order to elucidate structure-activity relationships. All the hexa-CB isomers induced a number of parameters of drug metabolism in chicks including cytochrome P-450, increased liver weight, and p-nitrophenyl glucuronyl transferase, but not

testosterone glucuronyl transferase activity. The most active inducers were the 2,3,4,2',3',4'- and 2,4,6,2',4',6'- isomers (Figures III.6.4 and III.6.5). The effects of 2,3,7,8-TCDF were both quantitatively and qualitatively different: it induced only a small increase in cytochrome P-450 and this cytochrome differed from that induced by the hexa-CBs, differing by a shift in the peak of the CO-difference spectrum from 450 nm to 449 nm and in characteristics of the ethyl isocyanide difference spectra (Table III.6.15). This is again important in showing that PCBs and PCDFs have independant toxic effects.

III.6.6 Other toxic effects in birds

Alteration of the thyroid has been reported in conjunction with PCB exposure. Hurst et al. (168) found depression of thyroid size, with low dosages or early in the experiment, followed by stimulation of thyroid growth yielding increased size with higher dose levels or later in the study. Increased thyroid weight and size in treated gulls were also reported by Jefferies and Parslow (173). These elevations did not appear to be dose-related and were accompanied by large, colloid-filled thyroid follicles. This condition parallels simple goiter resulting from insufficient intake or utilisation of iodine. Decreased mean heart weights were tabulated which would support the possibility of hypothyroidism suggested by the involution of the thyroid tissues and by a minimal decrease in secretion. Jefferies (174) has recently reviewed the role of the thyroid in the production of sublethal effects by PCBs and has suggested that effects on the thyroid may be linked with several other effects, including changes in vitamin A storage, decreased reproductive success and effects on behavior.

Cardiovascular effects of PCBs on birds were evaluated by Iturri et al. (175). Heart rate was reduced by Aroclors 1242 and 1254, but not affected by Aroclors 1221 or 1260. Aroclor 1254 lowered arterial blood pH significantly, which can lower cholinesterase activity.

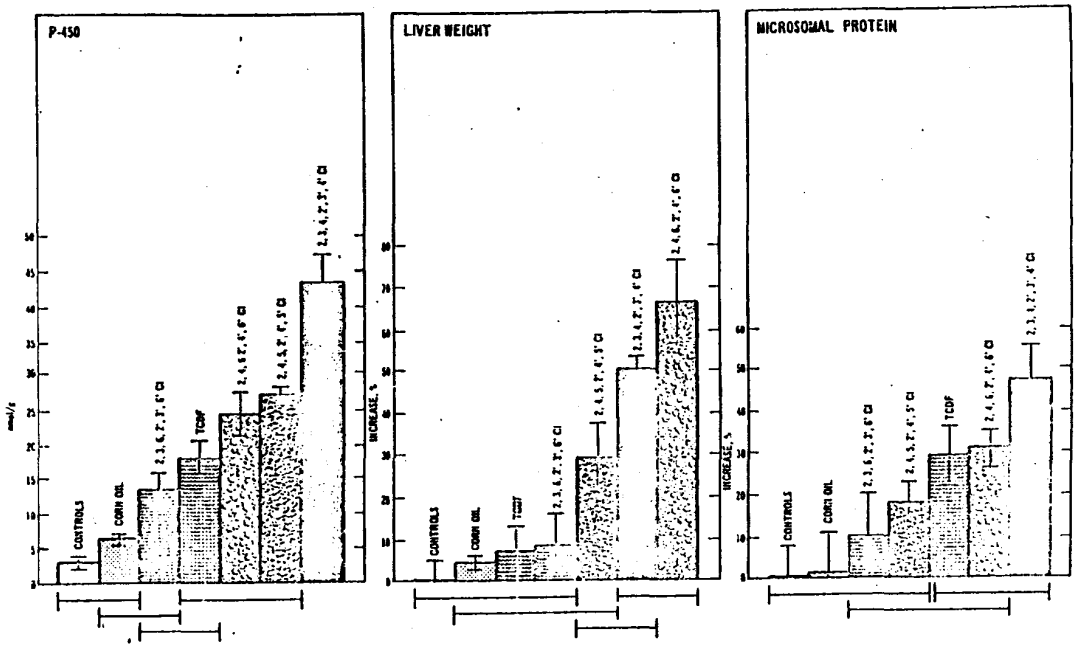

Figure III.6.4. *Effects of purified* hexachlorobiphenyl isomers and TCDF on chick livers (147). The heavy lines below the bar graph indicate groups which do not differ significantly from each other.

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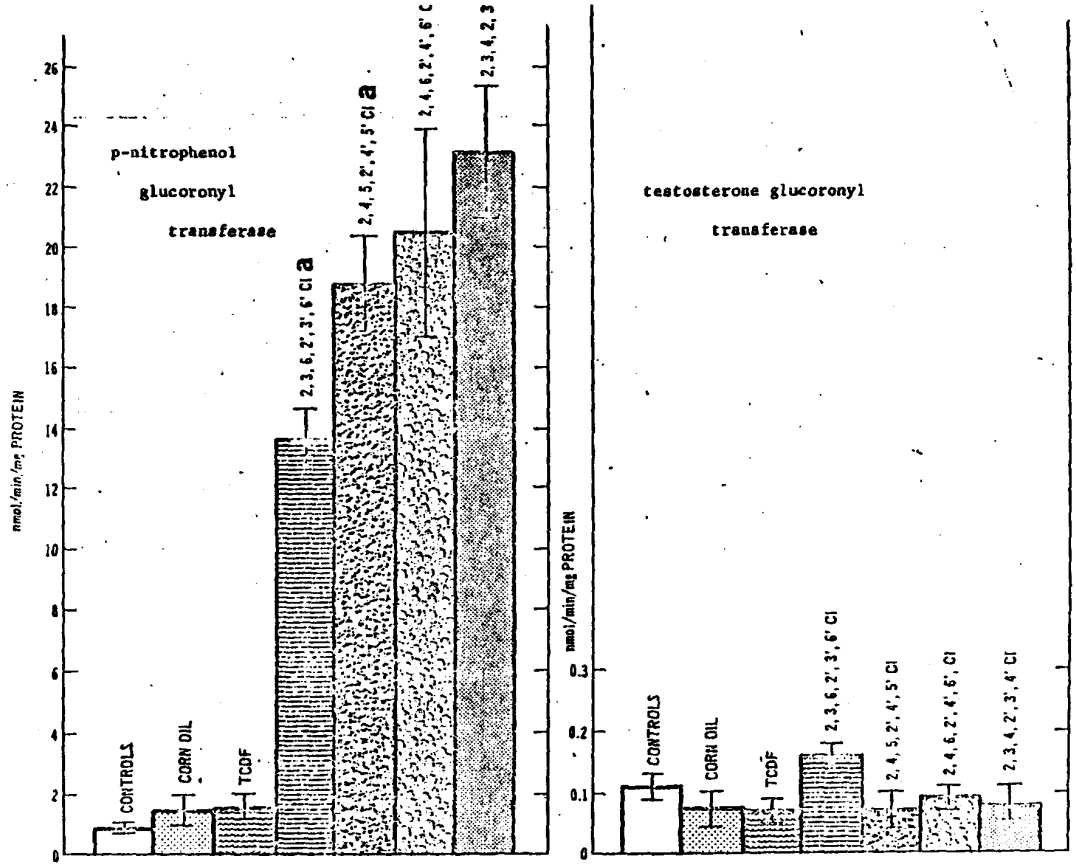

Figure III.6.5 (from ref. 147) Effects of hexachlorobiphenyl isomers and TCDF on chick liver enzymes.

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Table III.6.15 (from ref. 147)

EFFECTS OF HEXACHLORODIPHENYL ISOMERS AND TCDF ON ETHYL ISOCYANIDE DIFFERENCE SPECTRA IN THE CHICK[a]

TREATMENT	455/430 RATIO
Controls	0.42 ± 0.01
2,4,5,2',4',5'-HCB	0.40 ± 0.04
2,3,6,2',3',6'-HCB	0.40 ± 0.10
2,4,6,2',4',6',-HCB	0.37 ± 0.01
2,3,4,2',3',4'-HCB	0.43 ± 0.01
Corn Oil	0.51 ± 0.07
1 µg/kg TCDF	0.74 ± 0.06[b]

[a]Treatment is described in Figure 1. Values represent the means ± SE.

[b]Significantly different from controls, $P < 0.05$ (Duncan's Multiple-Range test).

Several authors have raported sublethal effects of PCBs on behavior. exposure of ring doves to 10 ppm Aroclor 1254 disrupted their in-ion behavior, which caused intermittent chilling of eggs and contributed ificantly to hatching failure (163). A similar phenomenon appeared to significant in American kestrels (164). Low doses of Clophen A50 (0.25 g/kg/day in the diet) are reported to have increased the nocturnal activity of migrating robins (176). Pheasant chicks hatched from hens fed 50 mg of Aroclor 1254 weekly made significantly more inappropriate choices in a visual cliff test than did controls, and were significantly easier to catch by hand in their pens (141, 159)*. Exposure to Aroclor 1254 also significantly impaired the escape responses of quail chicks when presented with strange objects (73). Although these behavioral effects may have little effect on the performance of birds in aviary situations, they have potentially large ecological significance for wild birds under natural environmental stresses.

Harris et al. (177) found a reduction in the size of the spleen and bursa of Fabricius in chicks hatched from chickens fed 10 and 20 ppm of Aroclors 1232, 1242, 1248, 1254 (Table III.6.16). As in earlier experiments (157) the effects of Aroclor 1016 were smaller than those of Aroclor 1242 and not statistically significant. An effect on the bursa of Fabricius was noted even at 5 ppm Aroclor 1248 (Table III.6.16) -- a level corresponding to a residue of only about 1.5 ppm of PCBs in the eggs (150). The bursa is the lymphoid organ responsible for establishing immunoglobin synthesis, but Harris et al. did not find a clear effect of PCB treatment on antibody production (177). However, Friend and Trainer (178) found that PCB-treatment increased the susceptibility of ducks to viral hepatitis.

* The effect on escape behavior was also marked when the male parent was treated, suggesting a possible mutagenic effect (141).

Table III.6.16 (from ref. 177)

Hatchability of eggs and bursa of Fabricius and spleen weights of chicks from hens fed Aroclors for 8 weeks (Experiment 1).

treatment (Aroclor[a])	PCB level (p.p.m.)	Hatchability of eggs (percent)	n	Progeny organ weights: bursa weight mg±SE	Progeny organ weights: spleen weight mg±SE
None	0	90	15	62.7 ± 4.0	17.0 ± 1.1
1232	5	90	5	54.1 ± 4.0	17.0 ± 0.6
	10	77[b]	5	59.2 ± 1.5	12.4 ± 0.8[b]
	20	51[b]	10	43.7 ± 3.5[b]	13.8 ± 0.8[b]
1016	5	83	5	49.0 ± 4.8	15.1 ± 1.1
	10	87	5	46.5 ± 3.2	13.8 ± 1.3
	20	92	5	77.8 ± 2.8	20.0 ± 0.7
1242	5	87	5	59.0 ± 9.6	16.4 ± 1.0
	10	46[b]	10	40.3 ± 3.2[b]	13.6 ± 0.9[b]
	20	4[b]	8	36.3 ± 2.5[b]	14.5 ± 1.2
1248	5	86	5	39.1 ± 3.3[b]	16.7 ± 2.3
	10	48[b]	10	42.5 ± 3.8[b]	13.5 ± 1.0[b]
	20	6[b]	5	36.3 ± 4.6[b]	13.6 ± 2.2[b]
1254	5	98	5	52.8 ± 1.6	15.3 ± 1.4
	10	88	5	48.7 ± 4.9	18.1 ± 1.4
	20	88	10	41.2 ± 2.9[b]	14.5 ± 0.7[b]

[a] Last two digits of Aroclor designate the % Cl, except Aroclor 1016 which contains 41% Cl.

[b] Significantly different ($p<0.05$) from the unsupplemented control using Student's "t" test.

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Combs *et al.* (397, 398) found that 10 ppm of Aroclor 1254 in the diets of female chickens increased the susceptibility of their offspring to vitamin E-selenium deficiency when the chicks were reared on a diet deficient in vitamin E and supplemented with a marginal level of selenium. Susceptibility to this deficiency, as measured by the incidence of exudative diathesis, was also increased when PCBs were added to chick diets. PCBs at 10 ppm in the diet caused a ten-fold increase in benzopyrene hydroxylase activity and this led to a decrease in the biological utilization of dietary selenium. The mechanism of the effect appeared to be oxidation of selenium, leading to reduction in its incorporation into glutathione peroxidase and its conversion into selenium compounds of limited biological usefulness (398).

III.7. Toxic Effects in Mammals: Acute and Subacute Studies

III.7.1. Acute toxicity

PCBs do not have high acute toxicity. Table III.7.1 summarizes measurements of the single-dose oral LD_{50} in rats and the single-dose dermal MLD in rabbits, for a range of Aroclor mixtures, as reported by the U.S. Food and Drug Administration (1). Weanling rats are more sensitive: the single-dose oral LD_{50}s for Aroclors 1254 and 1260 are 1,295 and 1,315 mg/kg (179). The single-dose i.v. LD_{50} for adult rats was 358 mg/kg (179). Grant and Phillips (180) obtained similar values for oral LD_{50} of Aroclor 1254 in 30-day-old rats (1,300-1,400 mg/kg) and higher values for 120-day-old rats (2,000-2,500 mg/kg).

The single-dose oral LD_{50} for Kanechlor KC-400 in mice was reported to be about 2,000 mg/kg. (181).

III.7.2. Subacute toxicity

Table III.7.2 summarizes reports of the subacute toxicity of PCBs available in 1972 (129, summarizing data from refs. 181-186). Together these data show that PCBs are toxic to mammals by ingestion, dermal absorption, and inhalation. Guinea pigs seem to be much more susceptible than rats and mice. Among signs of toxicity, liver damage is the most consistently observed (Table III.7.2). However, more thorough examination of rats killed by single doses of 4-10 g/kg of Aroclors 1254 and 1260 revealed hemorrhage into the lung, stomach and pancreas (187). Foci of ulceration surrounded by a severe inflammatory reaction were observed primarily in the duodenum and occasionally in the glandular part of the stomach.

TOXICITY OF AROCLORS*.

	Aroclors										
	1221	1232	1242	1248	1260	1262	1268	4465	5442	5460	2565
Oral LD_{50} mg/Kg (rats)	3980[a]	4470[a]	8650[a]	11,000[a]	10,000[b]	11,300[b]	10,900[b]	16,000[b]	10,600[b]	19,200[c]	6,310[c]
Skin MLD mg/Kg (rabbits)	>2000[a]	>1260[a]	>794[a]	>794[a]	>1260[b]	>1260[b]		>2000[b]	>1260[b]	>7940[c]	>2000[c]
	<3169[a]	<2000[a]	<1269[a]	>1269[a]	<2000[b]	<3160[b]	>2500[c]	<3160[b]	<2000[b]		<3160

[a]Undiluted.

[b]Administered as 50% solution in corn oil.

[c]Administered as 33.3% solution in corn oil.

*FDA Status Report on the Chemistry and Toxicology of Polychlorinated Biphenyls (PCB) or Aroclors as of June 1, 1970.

Table III.7.1 (ref. 2)

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Table III.7.2 (ref. 129)

Acute and Subacute Oral Toxicity Studies of PCB Preparations.

Preparation	Animal	Treatment	Mortality	Liver effects	References
Unknown	Mouse	single dose of approx. 2000 mg/kg	LD50		181
Aroclor 54% Cl	Rat	single dose of 500 mg/kg	0%	Increase of weight and lipid; potentiation of CCl_4 toxicity	182
Aroclor 42, 54, 60, and 68% Cl	Mallard	single dose of 2000 mg/kg	0%		183
42% Cl	Rat	20 daily doses of 138 mg	0% in 3 months	Hyalin bodies in liver cells	184
42% Cl	Guinea pig	2 doses of 69 mg 1 week apart	100% between 11 and 29 days	Fatty metamorphosis; central atrophy	184
68% Cl	Rat	6 daily doses of 300 mg	70% in 14 days	Increase of weight; cell swelling: hyalin granules	185
68% Cl	Rat	Doses of 50 mg every second day	60% in 3 weeks	23% weight increase; cell swelling; hyalin globules	185

Dermal Toxicity and Inhalation Studies of PCB Preparations.

Preparation	Animal	Treatment	Mortality	Liver effects	Skin effects	References
42% Cl	Guinea pig	11 daily skin applications of 34.5 mg	100% between 11 and 21 days	Fat; central atrophy; perinuclear basophilic granulation; focal necrosis in a few animals	Occasional thickening of the epidermis	184
42% Cl	Rabbit	Skin application at alternate days, total dose from 946 to 1980 mg	100% between 17 and 98 days	Fatty degeneration; central atrophy	Thinning of prickle cell layer and thickening of outer cornified layers	184
Aroclor	Rabbit	Daily skin applications of 0.3, 0.6, and 0.9 g	High dose died before liver necrosis developed	Moderate doses: mottled liver, subacute yellow atrophy, fatty degeneration, and marked necrosis	Reddening; formation of small papules and blisters; finally desquamation of external epidermal layers	186
Aroclor 68% Cl	Rat	Inhalation of 0.57 mg/cubic meter for 16 hours for 37 to 134 days	0%	Pale and yellow; cell swelling; hyalin degeneration; potentiation of CCl_4 and C_2H_5OH toxicity		185

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In recent studies of subacute toxicity, Allen and Norback (69) reported that rats fed 1000 ppm of Aroclors 1248, 1254, and 1262 died after 6-8 weeks due to widespread hepatic degeneration (188). After six weeks liver weights had increased from 2.8% of body weight in the controls to 9-10% of body weight in the treated animals. There was also marked atrophy of the thymus (188). Rats fed 1000 ppm of Aroclors 1254 and 1260 mostly died after 2-6 months with extensive liver damage (187); liver damage and a few deaths were observed also at 500 and 300 ppm (187, 189). In these cases the animals also suffered from severe pulmonary congestion and, in a few cases, from massive hemorrhages into the soft tissue or peritoneal cavity (187). Rats fed at 1000 ppm and 500 ppm of Kanechlors KC-500, 400 and 300 apparently had excess mortality within 25 weeks in conjunction with liver damage (190).

Mice fed 300 ppm of Aroclor 1254 for 6-11 months survived almost as well as controls but exhibited liver damage (191). Mice similarly survived well on a diet of 250 ppm KC-500, even in conjunction with BHC, but suffered liver damage (192). In comparative tests with three isomeric hexachlorobiphenyls (hexa-CBs) in male mice, the 28-day dietary LC_{50} was estimated to be over 300 ppm for 2,4,6,2',4',6'-hexa CB, 100-300 ppm for 2,4,5,2',4',5'-hexa CB, and only 86 ppm for 3,4,5,3',4',5'-hexa CB (193). In a subsequent test with 10 ppm of the last isomer, mice died in an average time of 39 days (193). The corresponding daily intake was about 2.1 mg/kg/day. In these experiments, atrophy of the thymus and spleen and porphyria were noted in addition to the liver effects

(193, 70 : Tables III.7.3 and III.7.4); these effects were especially marked with the exceptionally toxic isomer 3,4,5,3',4',5'-hexa CB.

Rhesus monkeys are considerably more sensitive to PCBs than rodents (69). Monkeys fed diets containing 100 and 300 ppm Aroclor 1248 developed facial edema, erythema, acne, and alopecia within 3 weeks. The lesions became progressively more severe with increased length of exposure. In addition, the animals developed anorexia, loss in weight, hypoproteinemia, hypolipidemia, and anemia. Within 3 months the majority of the monkeys were moribund. Necropsies of these animals revealed decided mucosal gastric hyperplasia with penetration of the glandular epithelium into the underlying submucosa (194). Numerous ulcerations of the hyperplastic gastric mucosa were also present (195). In addition, there was a decided hypertrophy of the liver.

Female rhesus monkeys fed 25 ppm Aroclor 1248 in the diet developed facial lesions similar to those observed in the animals receiving higher levels of PCBs (196). After two months on the PCB diet it was necessary to discontinue the exposure due to the severity of intoxication. One of the six experimental animals died 4 months after initial exposure to PCBs. Necropsy evaluation demonstrated severe gastric hyperplasia and ulceration, in addition to anemia, hypoproteinemia, bone marrow hypoplasia, and focal necrosis of the liver. The surviving adult female monkeys continued to be devoid of eyelashes and displayed facial acneform lesions 2 years following exposure to PCBs (69).

Rhesus monkeys appear to be even more sensitive to Aroclor 1242 than to Aroclor 1248. Although in the studies of Allen et al.

Table III.7.3 (from ref. 70)

Summary of the biological effects of hexachlorobiphenyl isomers in mice

Chemical	Dose level (ppm)	Mortality	Body weight gain (% of control)	Liver effect	Porphyrin Accumulation	Thymus effect
3,4,5,3',4',5'-HCB	10	0/5	12	++	+	++
	100	3/5	-	+++	+++	+++
	300	5/5	-	-	-	+++
2,4,5,2',4',5'-HCB	100	0/5	91	+	-	-
	300	1/5	58	++	-	±
2,4,6,2',4',6'-HCB	100	0/5	85	+	-	-
	300	5/5	-	++	-	++

Table III.7.4 (from ref. 70)

Summary of major pathological changes in mice given different hexachlorobiphenyl isomers for 28 days

Chemical	Dose level (ppm)	Liver	Thymus	Spleen	Heart
3,4,5,3',4',5'-HCB	30	Marked; fatty metamorphosis, single-cell necrosis.	Marked; involution.	Moderate; depletion of lymphocytes.	NS[a]
2,4,5,2',4',5'-HCB	300	Slight; swelling of hepatocytes.	Slight; involution.	NS[a]	NS[a]
2,4,6,2',4',6'-HCB	300	Marked; fatty metamorphosis, single-cell necrosis.	Marked; involution.	Moderate; depletion of lymphocytes.	Moderate; cardiomyopathy.

[a] NS = not significant.

MCNS 064335

rhesus monkeys survived and even produced a few young on diets of 5 and 2.5 ppm Aroclor 1248 (69), in studies by McNulty (197) all animals exposed to dietary levels of 100, 30, 10 and even 3 ppm of Aroclor 1242 died. The monkeys fed 3 ppm died after 8 months. The symptoms of poisoning were similar to those induced by Aroclor 1248, including cystic eyelids, loss of sebaceous glands, atrophy of the thymus, proliferation of the smooth endoplasmic reticulum of the liver, hyperplasia and ulceration of the gastric mucosa, with penetration of the epithelium into the submucosa (197, 198).

In tests with individual isomers, 2,4,4'-trichlorophenyl and 2,4,5,2',5'-pentachlorobiphenyl proved relatively non-toxic, causing no histopathological changes in 90 days at 10 ppm; for comparison, Aroclor 1242 at 10 ppm causes clinically detectable changes within 30 days and outspoken disease at 60 days. However, 3,4,3',4'-tetrachlorobiphenyl was very toxic and killed the test animal in less than 60 days (197). These preliminary experiments indicate a high degree of structural specificity for the toxic effects of chlorobiphenyls in monkeys.

Mink also appear to be extremely sensitive to PCBs (199, 200). Mink fed on a diet containing 30 ppm PCBs (10 ppm each of Aroclors 1242, 1248 and 1254) all died between the beginning of the breeding season and the end of the whelping period. Clinical signs and lesions included anorexia, bloody faces, fatty infiltration and degeneration of the liver and kidneys, and haemorrhagic gastric ulcers (199). In subsequent tests with Aroclor 1254, reductions in weight gain and

increased mortality were noted at dietary levels of 10 and 5 ppm; the effect of Aroclor 1254 was enhanced by simultaneous feeding of either DDT (10 ppm) or dieldrin (0.5 ppm) (Table III.7.5). All the mink fed 10 ppm of Aroclor 1254 and 0.5 ppm dieldrin died within 9 months (200). In a separate experiment, mink were fed on a diet containing beef from cows which had been fed with Aroclor 1254: the concentrations of PCBs in the total diet of two groups of mink were 3.57 and 0.64 ppm. All of the mink fed at the 3.57 ppm level died within 105 days, and 2 of 12 females fed at the 0.64 ppm level died at 122-129 days (201). Clinical signs of poisoning included yellowish discoloration of livers and blood in the gastro-intestinal tract or intra-abdominal hemorrhage (see also Table III. 6.6). The PCBs fed in this experiment appeared to be markedly more toxic to mink than those in the first experiment, in which the animals even on the 10 ppm dose were still growing after 4 months (Table III.6.5). This suggests the possibility that the toxicity of the PCBs had been enhanced during metabolism and storage in the cows.

Another species which appears to be extremely sensitive to PCBs is the big brown bat Eptesicus fucus. When fed only 10 ppm Aroclor 1254 big brown bats grew more slowly than controls and some individuals died (72).

No studies appear to have been reported of the acute or sub-acute toxicity of Aroclor 1016 in any species of mammal.

Table III.7.5

Effect of Aroclor 1254, Alone or in Combination with Pesticides, on Body Weight Gains of Female Mink

Dietary treatment	No. Mink	Initial body wt. (gm) (Aug. 25)	Body weight gain (gm) ± S.E. from initial wt. 1 mo. (Sept. 24)	2 mo. (Oct. 25)	3 mo. (Nov. 25)	4 mo. (Dec. 22)
Basal diet (30% ocean fish mix; control)	6	1020	43 ± 17.4	203 ± 25.4	187 ± 46.2	210 ± 35.1
Basal diet plus 5 ppm PCB[1]	6	940	62 ± 22.4	153 ± 44.2	138 ± 42.7	128 ± 43.7
Basal diet plus 10 ppm PCB	6	923	43 ± 20.4	148 ± 32.0	132 ± 26.3	92 ± 35.1*
Basal diet plus 10 ppm PCB and 10 ppm DDT	6	1052	43 ± 14.5	57 ± 14.6**	28 ± 21.5**	-15 ± 35.6**
Basal diet plus 10 ppm PCB and 0.5 ppm dieldrin	6	940	108 ± 25.1	133 ± 26.7	115 ± 18.9	57 ± 24.4**

(1) Aroclor 1254.
*Significantly different ($P < 0.05$) from controls.
**Significantly different ($P < 0.01$) from controls.

Effect of Aroclor 1254, Fed Alone or in Combination with Pesticides, on Mortality and Reproduction of Mink (Aug. 25 to May 10)
(6 females and 1 male/group)

	% mortality	Females alive	Females whelped	Kits born Alive	Kits born Dead	Kits/female alive
Control	14	5	3	17	8	5.0
5 ppm PCB[1]	29	4	0	0	0	0
10 ppm PCB	71	1	0	0	0	0
10 ppm PCB + 10 ppm DDT	43	4	0	0	0	0
10 ppm PCB + 0.5 ppm dieldrin	100	0	0	0	0	0

(1) Aroclor 1254.

MONS 064338

III.8. Toxicity of PCDFs in Mammals and the Role of PCDFs in the Toxicity of Commercial PCBs

Preliminary studies of the toxicity of 2,3,7,8-tetrachlorodibenzofuran (TCDF) in mammals have been reported by Moore et al. (71) and Goldstein et al. (146). In guinea pigs, the single-dose oral LD_{50} is between 5 and 10 μg/kg (Table III.8.1). The bottom of the table shows, for comparative purposes, that the LD_{50} of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) (2 μg/kg) compares quite closely with the TCDF value. TCDF toxicity in guinea pigs is one of a progressive weight loss followed by depression at least 24 to 36 hours preceding death. As was observed in the chicken toxicity studies, there was severe diminution in size of thymus and spleen. Similarly, this finding was found to correlate with a lymphocyte depletion in the periarterialor lymphocytic sheaths and follicles of the spleen; or severe atrophy of the thymus cortex. Although the numbers of animals evaluated were small, the trend of changes present in the bladder, adrenal, kidney, and liver were similar to that previously associated with TCDD toxicity (202). In contrast, TCDF appears to be much less toxic to rats and mice than to guinea pigs (71). A single experiment in which TCDF was administered to rats at doses up to 1,000 μg/kg failed to show any toxic effects. These rats were terminated 4 weeks after dosing. Histopathologic evaluation of tissues from the rats failed to reveal any changes that could be associated with tetrachlorodibenzofuran toxicity.

Table III.8.2 summarizes the TCDF toxicity findings in male mice. Per os dose levels up to and including 6,000 μg/kg in

Table III.8.2 (from ref. 71)

2,3,7,8-tetrachlorodibenzofuran toxicity in male C57 BL/6 mice[a]

Dose:	0 µg/kg	6,000 µg/kg
Mortality:	None	None
Body weight:	Normal gain	Sl. depression 2nd-5th day
Liver/body weight:	1.513 ± 0.05	1.728 ± 0.04
Ratio:	5.76 ± 0.05	6.86 ± 0.06
Thymus/body weight:	0.045 ± 0.002	0.017 ± 0.001
Ratio:	0.19 ± 0.01	0.06 ± 0.007

[a]Administered subcutaneously to 6-week-old mice. Animals sacrificed 30 days post-administration. "No effects" per. os, at 6,000, 4,000, 2,500, 1,500, 1,200, 1,000, 800, 600, and 400 µg/kg. LD_{50} 2,3,7,8-TCDD = 200-250 µg/kg.

Table III.8.1 (from ref. 71)

2,3,7,8-tetrachlorodibenzofuran toxicity in Hartley guinea pigs

Per os dose (µg/kg)	Mortality	Mean time to death
80	5/5	10.8 days
40	5/5	11.6
20	5/5	12.2
15	6/6	12.0
10	9/11	15.5
5	3/11	18.0
0	0/11	-0-

LD_{50} 2,3,7,8-TCDD = 2.0 µg/kg; MTD = 20.6 days.

a single dose failed to produce any observable effects during the ensuing 28 to 30 days. Histopathologic examinations of these animals failed to reveal any effects on thymus, spleen, or other organs, save for the liver. Here, a mild effect with the suggestions of a mild toxicity was observed in mice that received 6,000 μg/kg per os. A definite toxic response was observed in mice that received a 6,000 μg/kg dose administered subcutaneously. Although mortality was not observed, there was a depression in body weight gain during the second to fifth day following dosing. Organ weights recorded at time of necropsy 30 days after the dose had been administered showed an increase in the liver weight as well as liver to body weight ratio.

A more striking finding was the reduction in thymus weight with a concurrent reduction in thymus to body weight ratio. Histopathologic evaluations of tissues from these animals confirmed that the reduction in thymus weight was due to loss of lymphocytic elements. Evaluation of the liver evidenced a clear, but moderate, toxic response characterized by focal areas of single- cell necrosis and pleomorphism. The mouse studies show that there is at least a 30-fold diminution in TCDF toxicity when compared to TCDD. It further indicates, however, that the pattern of the toxicity will mimic that which is observed with TCDD (203).

Although 2,3,7,8-TCDF is the only CDF isomer whose toxicity has been investigated to date, it is expected to be the most toxic CDF, by analogy with the structurally similar 2,3,7,8-TCDD (148). It

is important to note that 2,3,7,8-TCDF is one of the CDFs that has been identified in Aroclors 1248 and 1254 (36).

The toxic effects of TCDF in mice are similar to those of 3,4,5,3',4',5'-hexa-CB (Tables III.7.3, III.7.4), but there are some important differences. The hexa-CB had a proportionately greater effect on the thymus and liver, and induced p-nitrophenyl glucuronyl transferase and ALA-synthetase, causing porphyria (146); as in the case of chicks (Figure III.6.4, Table III.6.7), TCDF did not have these effects in mice, even at comparable dose-levels (71, 146).

In a comparative study, Vos and Beems (204) found differences in toxicity between Aroclor 1260, Clophen A60 and Phenoclor DP-6 when applied to the skin of rabbits (118 mg PCB per rabbit 27 times in 38 days). Clophen A60 and Phenoclor DP-6 caused mortality (3/4 and 1/4 respectively) and severe skin lesions, whereas Aroclor 1260 caused no mortality and only slight skin lesions. All three mixtures caused liver damage, abdominal and pericardial edema, kidney lesions, and porphyria, but the liver damage was less severe in the Aroclor 1260-treated animals (Table III.8.3). Since PCDFs were known to cause skin lesions of this type, Vos attributed the difference in toxicity primarily to the PCDFs in the Clophen and Phenoclor mixtures (204, 129). However, in a subsequent experiment (205) both Aroclor 1260 and 2,4,5,2',4',5'-hexa-CB* caused liver injury, porphyria

* Some synthesized samples of 2,4,5,2',4',5'-hexa-CB are now known to be contaminated with 2,3,7,8-TCDF (206), but this does not appear to have been the case for the sample used in ref. 205.

Table III.8.3 (from ref. 204)

PATHOLOGIC FINDINGS IN LIVERS OF RABBITS TREATED WITH PCB FOR 38 DAYS, AND OF CONTROL ANIMALS[a]

	Phenoclor				Clophen				Aroclor				Control			
			[b]		[b]		[b]	[b]								
Fatty degeneration	2	2	1	1	1	1	1	—	—	—	1	2	—	—	—	—
Centrolobular loss of glycogen	—	1	2	1	2	1	1	2	—	—	1	—	—	—	—	—
Centrolobular degeneration	1	2	—	1	1	2	1	2	—	1	1	1	—	—	—	—
Focal hydropic degeneration	—	1	—	—	—	2	2	—	—	1	—	2	—	—	—	—
Focal necrosis	—	1	—	—	2	2	2	2	—	—	1	2	—	—	—	—
Centrolobular liver cell atrophy	2	1	1	2	2	1	2	1	—	—	—	1	—	—	—	—
Enlargement of nuclei	2	1	—	1	2	2	2	2	1	—	1	2	—	—	—	1
Cytoplasmic hyalin degeneration	1	—	1	2	2	1	2	2	—	—	—	1	—	—	—	—
Ceroid pigment in Kupffer cells	—	1	—	2	1	1	1	1	—	2	1	2	—	—	—	—
Periportal fibrosis	2	—	2	1	1	1	2	2	1	—	1	1	—	—	—	—

[a] Severity of lesion: 1 = slight, 2 = marked.
[b] Animals that died during the experiment.

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and slight skin lesions. Vos (129) concluded that both PCDFs and PCBs cause liver damage and skin effects, but that PCBs are the primary cause of porphyria (Table III.8.4). This conclusion has been confirmed by subsequent experimentation (70, 71, 146, 147, 193), but several experiments have suggested further that PCBs play some role in the induction of edema and hydropericardium also (Section III.6.2 above, 204).

McNulty (197) has discussed the possibility that PCDFs might be responsible for the extreme toxicity of PCBs in rhesus monkeys. In a preliminary test, 2,3,7,8-TCDD was lethal to a rhesus monkey after feeding for two months at 2 ppb in the diet -- i.e., it was about 5,000 times as toxic as Aroclor 1242 under the same chronic feeding conditions. TCDD is usually 4 to 10 times as toxic as TCDF (71, 129): hence it is unlikely that TCDF will prove to be more than 1,000 times as toxic as Aroclor 1242. Accordingly it is very unlikely that the presence of PCDFs at 1 or 2 ppm in Aroclor 1242 could account for its toxicity to monkeys (197). McNulty suggested the possibility that PCDFs might be produced by metabolism in monkeys (197). The relative roles of PCBs and PCDFs in causing skin lesions in humans will be discussed further below.

In summary, 2,3,7,8-TCDF is extraordinarily toxic to guinea pigs and to chickens, but not to mice or rats. There are both parallels and differences between the toxic effects of PCBs and PCDFs. Some, but not all, of the toxic effects of PCB mixtures can be attributed to contamination with PCDFs. Since PCDFs are present in

Table III.8.4 (from ref. 129)

Probable Contribution of Polychlorinated Dibenzofuran (PCF) and Pure Polychlorinated Biphenyl (PCB) in the Toxicity of Crude PCB Mixtures.

	Chloracne	Edema formation	Liver damage	Hepatic porphyria
Polychlorinated Dibenzofuran	++	++	++	–
Polychlorinated Biphenyl	+	–	+	++

commercial mixtures (Section II.5.5) and appear to be formed from them in the environment (Section II.8.3), in service (Section II.8.4), and by metabolism (Appendix E), it is difficult and probably academic to attempt to distinguish their effects completely in weighing the environmental hazards posed by PCBs.

III.9 Chronic Effects of PCBs in Mammals and Effects on Reproduction

This section summarizes the results of long-term chronic toxicity tests with PCBs in mammals and the gross effects observed, including effects on reproduction. Discussion of biochemical effects is reserved to Sections III.10 and III.11 and discussion of carcinogenic effects to Section III.14.

III.9.1 Effects on rats

In a study reported by Linder et al. (179), Sherman rats were exposed for two generations to dietary levels of 1, 5, 20 and 100 ppm Aroclor 1254, and 5, 20 and 100 ppm Aroclor 1260. Exposure of the F_0 generation started at 3-4 weeks of age and continued through mating, gestation and lactation. Each generation was mated twice. Rats exposed to dietary levels of 20 ppm (1.5 mg/kg/day) or more had fewer pups per litter than controls in the F_{1b} and F_2 generations. The 100 ppm exposure level of Aroclor 1254 increased mortality in the F_{1b} offspring and markedly decreased mating performance of the F_{1b} adults (Table III.9.1). The 500 ppm dietary level of Aroclor 1260 reduced litter size and decreased survival in the F_1 litters. Dietary levels of 5 ppm Aroclor 1254 and 100 ppm Aroclor 1260 had no

Table III.9.1 (from ref. 179)

Reproduction and pup survival in groups of rats fed Aroclor 1254

Generation	Dietary level (ppm)	Parental exposure (days)†	No. of females mated	No. of litters		Litter size§		Total pups/group				Survival (%)	Mean body weight at weaning (g)
								Born (found)		Alive at			
				Born‡	Weaned	At birth	At weaning	Dead	Alive	Day 3	Weaning		
F_{1a}	0‖	67	10	8	7	11·1	10·6	3	89	86	85	95·5	39·2
	100‖	67	10	9	8	9·4	8·1	6	85	78	73	85·9	31·4
	500‖	67	10	4 (2)	0	4·0*	—	5	8	0	0	0	—
	0	62	20	17	17	12·4	11·8	0	211	205	201	95·3	38·7
	20	62	20	19	19	11·7	11·5	3	222	220	219	98·6	38·3
	100	62	20	19	19	10·7*	10·3	10	204	199	196	96·1	32·9
	0	67	20	18	18	11·2	11·1	2	202	200	200	99·0	38·5
	1	67	20	16	15	9·2	9·1	3	147	145	145	98·6	42·3
	5	67	20	17	17	10·8	10·8	1	184	184	183	99·5	42·1
F_{1b}	0‖	186	10	7	7	11·6	11·6	1	81	81	81	100	37·9
	100‖	186	10	8	6	11·8	8·0	0	94	74	64	68·1**	27·9
	0	188	20	17	17	12·3	11·1	2	209	194	189	90·4	37·1
	20	188	20	18	18	10·4*	10·1	1	188	187	181	96·3	39·0
	100	188	20	20	19	9·5**	7·0	6	189	164	139	73·6	32·1
	0	201	20	18	18	12·3	11·0	6	222	211	198	89·2	40·7
	1	201	20	18	17	11·1	9·8	3	200	194	176	88·0	38·0
	5	201	20	20	19	11·1	10·2	2	222	218	203	91·4	38·9
F_{2a}	0	129	20	18	18	12·5	12·2	0	225	221	220	97·8	35·5
	20	129	20	17	17	10·6*	10·1	5	181	174	171	94·5	36·8
	100	129	20	7 (2)	4	7·2**	5·6	7	36	29	28	77·8**	35·2
	0	125	20	19	19	11·7	11·5	3	223	220	218	97·8	37·4
	1	125	20	15	15	11·7	11·5	0	175	174	173	98·9	37·2
	5	125	19	17	17	12·1	11·7	1	205	199	198	96·6	35·7
F_{2b}	0	274	20	17	16	12·7	11·3	4	216	201	191	88·4	31·9
	20	274	20	12	12	9·6**	8·5	6	115	103	102	88·7	36·1
	100	274	20	4 (2)	2	3·5**	3·5	7	7	7	7	100·0	38·3

† Conception to mating for the parents of the F_{2a} and F_{2b} generations.
‡ Numbers in parentheses indicate numbers of litters in which no live offspring were found.
§ Number of live offspring/live litter born.
‖ One-generation study.
Values marked with asterisks differ significantly from the control value: * $P < 0.05$; ** $P < 0.005$.

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effects on reproduction. Liver weights were markedly increased in 21-day-old male weanlings at the 1 ppm level of Aroclor 1254 and in both sexes in both generations at 5 ppm or higher levels of Aroclor 1260 (Table III.9.2).

In this study rats were killed when their offspring were weaned (after 180-330 days' exposure) (179). In this and in earlier studies in which rats were exposed to Aroclor 1254 and 1260 for periods of 6 to 8 months (187, 189), the principal pathological lesions observed were in the liver. These included hypotrophy, brown pigment in Kupffer cells, adenofibrosis and hyperplastic nodules (179, 187, 189). Adenofibrosis and nodules were observed principally at the higher levels of exposure and were most frequent in the second generation animals exposed to Aroclor 1254 (Table III.9.3). Liver nodules were even seen in F_{1b} rats exposed to 20 ppm Aroclor 1254 (ibid.). For further discussion see below under Carcinogenesis and Appendix D.

In another three-generation reproduction study in rats involving exposure to Aroclors 1242, 1254, and 1260, reproduction was unaffected in the first generation, but was adversely affected at dietary levels of 10 and 100 ppm of all three Aroclors in the second and third generations (68). No adverse effects were reported at 1 ppm. The cause of the adverse effects was a decrease in mating indices and in the incidence of pregnancy (68). Other aspects of reproduction are said to have been unaffected, but no details have been given, and in earlier reports on this study decreased survival of offspring was reported at 100 ppm of Aroclors 1242 and 1254 (1, p. 142). In a concomitant 2-year study of rats exposed to the same three Aroclor

Table III.9.2 (from ref. 179)

Mean liver weights of 21-day-old rats from parents fed Aroclor 1254 or 1260

Aroclor	Generation	Dietary level (ppm)	Liver weight			
			Males		Females	
			g	% of body weight	g	% of body weight
1254	F_{1a}	0	1·40	3·69	1·38	3·83
		1	1·81*	4·01*	1·63	3·91
		5	1·92*	4·34*	1·86*	4·43*
	F_{1b}	0	1·58	3·69	1·58	3·84
		1	1·54	3·94*	1·49	3·92
		5	1·77	4·63*	1·72	4·75*
	F_{2a}	0	1·37	3·81	1·28	3·74
		20	2·26*	5·78*	2·23*	6·08*
		100	2·63*	6·97*	2·67*	7·10*
	F_{2b}	0	1·63	3·78	1·42	3·63
		1	1·49	3·74	1·36	3·69
		5	1·56	4·23*	1·38	4·09*
1260	F_{1a}	0	1·54	3·66	1·57	3·92
		5	1·73	4·18*	1·70	4·39*
	F_{1b}	0	1·59	3·73	1·55	3·93
		20	2·06*	5·04*	2·14*	5·18*
		100	2·59*	6·33*	2·59*	6·48*
	F_{1b}	0	1·35	3·59	1·29	3·70
		5	1·58	4·01*	1·52*	4·07*
	F_{2a}	0	1·65	3·82	1·64	4·02
		20	2·06*	5·21*	1·94	5·06*
		100	2·50*	6·12*	2·49*	6·85*
	F_{2a}	0	1·41	3·85	1·36	3·87
		5	1·66*	4·21*	1·60*	4·25*

Values marked with an asterisk differ significantly from the control value: $^*P < 0{\cdot}05$.

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Table III.9.3 (from ref. 179)

Body weight, liver weight and liver pathology of F_0 and F_{1b} adult rats fed Aroclor 1254 or 1260

Generation	Sex	Exposure (days)	Dietary level (ppm)	Terminal body weight (g)	Liver weight		No. of livers examined	Liver pathology (no. of livers affected)						
					g	% of body weight		Enlarged hepatocytes	Inclusions	Foamy cytoplasm	Pigment	Fibrous strands	Adenofibrosis	Nodules
								Aroclor 1254						
F_0	M	310	0	601	15·22	2·53	10	0	0	0	0	0	0	0
			1	602	15·88	2·60	10	0	0	0	0	0	0	0
			5	614	15·37	2·51	10	0	0	1	2	0	0	0
	F	313	0	370	10·34	2·80	10	0	0	0	1	0	0	0
			1	358	10·14	2·84	10	1	0	0	4	0	0	0
			5	362	10·66	2·94	10	4	1	0	3	0	0	0
F_{1b}	M	328	0	588	14·51	2·47	10	0	0	0	0	0	0	0
			20	602	16·11*	2·67*	8	8	3	7	1	0	0	0
			100	554	19·43*	3·54*	10	10	5	7	4	2	3	1
	F	328	0	349	9·92	2·85	10	0	0	0	0	0	0	0
			20	340	9·41	2·78	7	7	2	5	7	0	0	3
			100	300*	10·53	3·60*	10†	10	2	8	9	3	5	7
F_{1b}	M	190	0	475	11·13	2·34	10	0	0	0	0	0	0	0
			1	453	11·14	2·44	10	0	0	0	0	1	0	0
			5	495	12·19	2·46	10	2	0	2	0	1	0	0
	F	190	0	287	8·27	2·87	10	0	0	0	0	0	0	0
			1	294	8·44	2·86	10	1	1	0	0	4	0	0
			5	299	8·92	2·98	10	1	0	0	0	1	0	0
								Aroclor 1260						
F_0	M	250	0	563	14·45	2·57	10	1	0	0	0	0	0	0
			5	567	15·06	2·65	10	3	0	2	0	0	0	0
	F	250	0	322	10·09	3·13	10	0	0	0	0	0	0	0
			5	331	10·80	3·25	10	1	0	0	0	0	0	0
F_{1b}	M	179	0	472	12·68	2·68	10	0	0	0	0	0	0	0
			20	494	14·72*	2·99*	10	8	5	4	0	0	0	0
			100	509	16·63*	3·27*	10	10	10	3	0	0	0	1
	F	179	0	297	9·66	3·27	10	0	0	0	0	0	0	0
			20	304	10·30	3·40	10	6	0	0	0	0	0	0
			100	304	11·14*	3·70*	10	9	0	1	5	2	1	2
F_{1b}	M	217	0	492	11·97	2·43	10	0	0	0	0	0	0	0
			5	505	13·16	2·60*	10	7	2	2	1	0	0	0
	F	217	0	279	7·93	2·84	10	0	0	0	0	0	0	0
			5	286	8·49	2·96	10	2	0	0	0	0	0	0

† Necrosis in two livers.

Values marked with an asterisk differ significantly from the control value: * P 0.05.

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mixtures, the most noteworthy effects were increases in liver weight and pathological changes in the liver (68, 207: for further discussion see below under Carcinogenesis and Appendix D).

Sherman rats exposed to 100 ppm of Aroclors 1016 and 1242 for 4-10 months also showed a variety of microscopic changes in their livers (208). These changes were somewhat less severe than those observed in animals fed 100 ppm of Aroclors 1254 and 1260 (Tables III.9.4, III.9.5), in that the animals fed Aroclors 1016 and 1242 did not show the lipid accumulation pronounced in the other groups. The changes were slightly, but not markedly, less severe in the rats fed Aroclor 1016 than in those fed Aroclor 1242 (Table III.9.4).

In a short-term (90-day) oral toxicity study with Aroclor 1016, liver weights were significantly increased at dietary exposure levels of 300 ppm, but not at 100 or 30 ppm (209). The study was too short, however, to provide a useful comparison with that in ref. 207, in which no changes in liver weight were reported in rats exposed to 100 ppm Aroclor 1242 for 3 months. However, in another study, the liver weights of rats were markedly increased by oral dosage of only 1 mg/kg (equivalent to about 15 ppm in the diet) for only 21 days (Table III.10.5): the effects of Aroclors 1016 and 1242 were similar (243). In another study, rats exposed to 100 ppm Aroclor 1242 for 2 months showed a significant increase in liver weight and a marked decline in liver vitamin A (166). Aroclor 1242 also had estrogenic effects in female rats (166), and dietary exposure to 30 or 100 ppm Aroclor 1016 markedly reduced gonad weights in female rats (209).

Table III.9.4 (ref. 208)

Incidence of Microscopic Changes Observed in Livers of Rats Fed Aroclor 1242 or 1016

Length of Exposure	Enlarged Hepatocytes		Vacuolated Cytoplasm		Inclusions		Brown Pigment		Foamy Cytoplasm		Foci of Necrosis Hemorrhage	
	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016	Aro 1242	Aro 1016
4	2/4	4/4	...	1/4	...	...	...	...	...	...	...	1/4
6	4/4	4/4	3/4	1/4	2/4	3/4	...	...	...	...	...	...
8	4/4	3/4	3/4	...	3/4	...	2/4	...	...	...	1/4	...
10	4/4	4/4	4/4	1/4	1/4	...	2/4	...	2/4	...	...	...
6 mo exp 2 mo rec	2/4	1/4	3/4	1/4	1/4	...	...	...	...	...	...	...
6 mo exp 4 mo rec	3/3	3/3	1/3	1/3	1/3	1/3	...	...	...	...	...	...
8 mo exp* 6 mo rec	3/4	...	...	...	2/4	...	...	...	...	...	...	...

* Only rats fed Aroclor 1242 were examined microscopically.

Table III.9.5 (ref. 187)

—Light Microscopic Findings in the Liver of Rats Fed Aroclor 1260*

Dietary Level (ppm)	Sex	Enlarged Hepatocytes	Inclusions in Cytoplasm	Adenofibrosis	Increased Lipid	Foamy Cytoplasm	Pigment
0	M	...	...	...	...	...	...
20	M	10	9	...	...	3	...
100	M	10	9	...	10	...	...
500	M	10	4	...	10	7	1
1,000	M	10	6	2	10	9	2
0	F	...	...	...	...	...	...
20	F	3	...	...	...	...	...
100	F	7	2	1	10	...	1
500†	F	9	5	1	9	8	6
1,000‡	F	6	...	4	7	4	2

* In their diet for eight months; ten rats/group. † Only nine animals were studied. ‡ Only seven animals were studied

—Light Microscopic Findings in the Liver of Rats Fed Aroclor 1254*

Dietary Level (ppm)	Sex	Enlarged Hepatocytes	Inclusions in Cytoplasm	Adenofibrosis	Increased Lipid	Foamy Cytoplasm	Pigment
0	M	...	...	...	...	...	...
20	M	7	1	...	...	1	...
100	M	10	7	1	10	10	3
500	M	10	2	10	10	4	2
0	F	...	...	...	...	...	...
20	F	...	...	...	...	...	1
100	F	10	7	7	10	9	10
500†	F	9	2	9	9	2	7

* In their diet for eight months; ten rats/group.
† Only nine tested since one rat that died showed too much autolysis.

III.9.2 Effects on mice

In chronic feeding studies with mice, the most noteworthy effects were on the liver (see below under Carcinogenesis and Appendix D). Daily dosage of mice with 1 ml KC-400 caused effects on the skin (loss of hair, erosion and ulceration after 3 months) in addition to enlargement of the liver and other pathologic changes (210).

No chronic studies of effects on reproduction have been reported, but a single dose of 20 mg/kg Clophen A60 lengthened oestrous cycles in mice (211).

III.9.3 Effects on dogs

A two-year chronic feeding study was conducted in beagle dogs exposed to 1, 10, and 100 ppm of Aroclors 1242, 1254 and 1260 in the diet (68, 212). Four dogs of each sex were included in each of the 9 feeding groups and in a tenth control group; the dogs were 4-7 months old at the start of exposure. Six dogs died during the experiment: of these three females died from chronic peritonitis (one on 100 ppm Aroclor 1242, one on 10 ppm Aroclor 1254, and one on 100 ppm Aroclor 1260). Table III.9.6 summarizes the principal findings at the end of the two-year exposure period: there were dose-related increases in liver weights, serum alkaline phosphatase levels, and leukocyte counts. The most noteworthy findings on gross pathologic observation were "numerous pinpoint nodules" in the stomachs of dogs in all treatment groups (except the 1 ppm Aroclor 1242 group). These nodules were observed in 22 of the 66 treated dogs,

Table III.9.6. Effects in dogs after two-year oral exposure to PCBs (from data in ref. 212)

	Leukocyte counts (thousands/mm^3)	Serum alkaline phosphatase KA units/100 ml	Liver weight, % of body weight	Pinpoint nodules in stomach
Controls (8)	11.45	5.22	3.51	0/8
Aroclor 1242:				
1 ppm (8)	12.44	4.48	3.26	0/8
10 ppm (7)	13.46*	5.93	3.62	3/7
100 ppm (7)	19.93***	5.91	3.87	4/7
Aroclor 1254:				
1 ppm (8)	14.26**	5.62	3.52	3/8
10 ppm (7)	14.19**	7.49	3.64	1/7
100 ppm (7)	20.59***	9.64**	4.11*	5/7
Aroclor 1260:				
1 ppm (8)	14.28	5.77	3.77	2/8
10 ppm (8)	14.69**	4.98	3.73	2/8
100 ppm (6)	12.11	30.22***	5.03**	2/6

*, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$; significant differences from controls.

versus none in the 8 controls. No histopathological diagnoses of these lesions were included in the original report (212), but in 1975 it was reported that re-examination of the slides had shown evidence of "gastrointestinal inflammatory lesions and ulcerations which appeared similar to those reported by Allen (69) in rhesus monkeys".* Six of the dogs with stomach lesions, and two of the others, also had inflamed kidneys. The stomach lesions were equally prominent in dogs fed all three Aroclors, but the effect on liver weight was greatest in those fed Aroclor 1260 (Table III.9.6).

III.9.3 Effects on Non-human Primates

Rhesus monkeys are extremely susceptible to the effects of PCBs (69). Female monkeys fed 25 ppm Aroclor 1248 in the diet developed severe facial lesions and alopecia and had to be removed from the contaminated diet after 2 months. The five surviving females were still devoid of eyelashes and had facial acneform lesions two years later. Although several of these females conceived, all but one appeared to experience either fetal resorption or abortion. The one infant born was underweight (375 g versus 544 g) and had high levels of PCBs in the fat (28 ppm) and adrenals (24 ppm) (196).

Female monkeys given 2.5 and 5.0 ppm Aroclor 1248 in their diets developed facial edema, swollen eyelids, erythema, loss of hair, and acne within 2 months (213). By the fourth month, irregularities in the menstrual cycles and an increased level of urinary ketosteroids

* This statement, made at the National Conference (9), was deleted from Calandra's published paper (68), which states that "No remarkable histopathologic changes were found".

were recorded (214). Following 6 months of PCB exposure the female monkeys were bred to control males. Six of eight animals on the 5.0 ppm diet conceived (Table III.9.7). The remaining two were bred on five separate occasions without conceiving. Four of the six females experienced abortion early in gestation. Eight of eight of the 2.5 ppm PCB-fed animals conceived; however, only five were able to carry their infants to term. As was the case with infants of animals given the higher levels of PCBs, all the infants were small and at birth their skin contained detectable levels of PCBs.

The infants were permitted to nurse their mothers for 4 months.* Within 2 months focal areas of hyperpigmentation, swollen lips and eyelids, loss of eyelashes, and acneform lesions of the face developed. The skin of these infants showed a decided increase in the PCB level over this period. Within 4 months, 3 of the 6 infants died due to PCB intoxication. After weaning, the remaining three have shown improvement of the skin lesions during the 4-month period.

Earlier studies by this group have shown that infant rhesus monkeys are susceptible to other effects of PCBs. Month-old rhesus monkeys dosed with 35 mg/kg Aroclor 1248 for only four weeks suffered, in addition to skin lesions, atrophy of the thymus, edema and hyperplasia of the gastric mucosa, enlargement of the liver with proliferation of the smooth endoplasmic reticulum, and an increase in the RNA/DNA ratio in liver (214). Adult rhesus monkeys displayed hyperplasia and ulceration of the gastric mucosa after a single dose of 1.5 g Aroclor 1248 (215).

* The mothers' milk contained 0.15-0.40 ppm of PCBs (69).

Table III.9.7 (from ref. 69)

Modification in reproduction in primates that were exposed to Aroclor 1248 in the diet

	Control	2.5 ppm	5.0 ppm
Total impregnated (no./no. animals)	12/12	8/8	6/8
Resorptions or abortions (no./no. animals)	0/12	3/8	4/8
Stillborn (no./no. animals)	0/12	0/8	1/8
Normal births (no./no. animals)	12/12	5/8	1/8

Male monkeys appear to be less susceptible than females to reproductive dysfunction by PCBs (69). Four adult male rhesus monkeys were also exposed to a diet containing 5.0 ppm Aroclor 1248 for 17 months (average total intake of PCBs 460 mg). They began to develop a slight periorbital edema after 6 months of exposure; however, it was much less severe than in the female monkeys receiving a similar level of PCB. The morphological features and viability of the spermatozoa as well as the ability to fertilize control female rhesus monkeys was unaffected during the initial 12 months of PCB exposure. Subsequently one of the four males lost weight and developed alopecia, acne, periorbital edema and decreased libido. A testicular biopsy of this animal showed a decided hypoactivity of the seminiferous tubules. There was an absence of mature spermatozoa and a predominance of Sertoli cells of the tubules. The remaining three males have remained healthy and sexually active (69).

III.9.4 Effects on Mink

Mink also appear extremely sensitive to the effects of PCBs. In tests with Aroclor 1254, most females died and none produced young when fed diets containing 15 or 10 ppm (199, 200). In one test at 5 ppm no young were produced, and in a second test only 3 young were born alive from 12 exposed females (Table III.9.8). Reproduction was also reduced at 1 ppm Aroclor 1254, because one female died and two others produced no young (Table III.9.8). In another experiment in which mink were fed a diet containing 0.64 ppm of PCB residues in beef resulting from feeding PCBs to cows, only one of ten exposed

Table III.9.8 (from ref. 200)

Effect of Aroclor 1254 Fed Singly and in Combination with Hydrocarbon on Reproductive Parameters (Jan. 1 to May 10) (12 females and 4 males/group)

	% mortality	Females alive	Females whelped	Kits born Alive	Kits born Dead	Kits/female alive
Control (ocean fish)	6	11	11	56	10	6.0
Raw coho salmon	19	11	0	0	0	0
Cooked coho salmon	6	12	0	0	0	0
1 ppm PCB(1)	5*	10	8	35	8	4.3
5 ppm PCB	0	12	3	3	6	0.8
15 ppm PCB	31	8	0	0	0	0
5 ppm PCB + 6 ppm DDT	0	12	4	5	6	0.9
5 ppm PCB + 6 ppm DDT + 0.2 ppm dieldrin	19	10	5	15	7	2.2

(1) Aroclor 1254.
* One female escaped.

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females produced a litter: three kits were born to this female but they died during their first day of life (201). Thus, as already noted in Section III.7 above, the toxicity of the PCBs appears to have been enhanced during their metabolism and storage in the cows.

III.9.5 Effects on Swine

Hansen et al. (216) reported an experiment in which sows were fed a diet containing 20 ppm Aroclor 1242 from three weeks prior to conception throughout gestation and nursing. One of five treated sows failed to conceive and the other four produced significantly fewer live young than controls, due to an excess of stillbirths and fetal deaths (Table III.9.9). Treated sows had enlarged livers and their offspring had unusually small spleens and thyroids (Table III. 9.10). The principal lesions observed in both sows and offspring were erosions in the gastric mucosa. A number of the treated offspring, but no controls, died from chronic septicemia, suggesting that the exposure to PCBs had increased their susceptibility to infection. These adverse effects were associated with residues of only 4-20 ppm in the fat of the sows, and less than 2 ppm in their milk (214).

III.9.6 Effects on rabbits

Oral administration of a single dose of 12.5, 25, or 50 mg/kg of Aroclor 1254 to pregnant rabbits had embryotoxic effects, but single doses of 10 and 1 mg/kg did not (217, 218).

Table III.9.9 (from ref. 216)

Farrowing Records and Polychlorinated Biphenyl (PCB) Residues of Sows Fed PCB Mixture in Ration and of Nontreated Control Sows

Farrowing weight (kg)	Total PCB (ppm) Blood	Total PCB (ppm) Fat	Offspring: Live No.	Offspring: Live Mean body weight (kg)	Offspring: Stillborn	Offspring: Fetal deaths*
Control sows						
288	0.001	0.1	13	1.1	5**	0
277	0.001	0.1	12	1.5	0	0
295	0.001	0.1	10	1.8	1	0
259	0.001	0.1	11	1.5	0	0
284	0.001	0.1	11	1.5	1	1
Treated sows						
268	0.096	4.1	11	1.3	2	0
294	0.192	11.8	10	1.5	1	1
270	0.383	19.5	6	1.4	1	3
289	....	8.1	5	1.5	0	1
286†	0.337	11.7	....	....	....	....

* Mummified, between (2.5 cm to near term) from examination of placenta. ** Last 5 in litter of 18; delivered 28 to 36 hours after 1st pig. † Fed control ration for 5 weeks before slaughter; this sow did not conceive.

Table III.9.10 (from ref. 216)

Organ Weight to Body Weight Ratios for Sows Given PCB Mixture in the Feed, for Nontreated Control Sows, and for Their Offspring

Organ	Organ weight to body weight ratio (mean × 10^2 ± se): Sows: Control	Sows: Treated	Offspring: Control	Offspring: Treated
Liver	11.37 ± 0.08	14.57 ± 1.08	24.52 ± 0.97	25.20 ± 1.70
Kidney	1.71 ± 0.32	2.02 ± 0.40	4.08 ± 0.35	4.38 ± 0.79
Spleen	1.09 ± 0.08	1.19 ± 0.19	1.72 ± 0.24	1.47 ± 0.13
Heart	2.64 ± 0.40	2.63 ± 0.22	4.82 ± 0.31	4.60 ± 0.35
Brain	0.70 ± 0.40	0.87 ± 0.08	3.55 ± 0.44	3.70 ± 0.34
Thyroid gland	0.086 (n = 1)	0.080 ± 0.008	0.111 ± 0.018	0.073 ± 0.006
Adrenal gland	0.070 ± 0.008	0.088 ± 0.008	0.080 ± 0.010	0.094 ± 0.012

n = Number of sows; se = standard deviation.

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III.10. Enzyme Induction and Other Effects on the Liver

Some of the most general and significant sublethal affects of PCBs in mammals are on the liver and on hepatic enzymes. This section reviews these effects, with the exception of chemical porphyria (Section III.11) and carcinogenesis (Section III.14).

III.10.1. Effects on liver weight

An increase in liver weight and/or in liver-to-body weight ratio has been noted in many studies. Litterst at al. (219) observed increased liver-to-body weight ratios in rats fed 50 and 500 ppm of Aroclors 1242, 1248, 1254 and 1260 for only 4 weeks (Table III.10.1). Similar increases in liver weight have been noted in long-term feeding studies with these mixtures in rats (69, 166, 179, 207). In general the effects were greater with the more chlorinated mixtures (Table III.10.1, 207), but in an experiment with Aroclor 1016 marked effects were seen in only 21 days after oral dosage of only 1 mg/kg/day (equivalent to about 12 ppm in the diet) (Table III.10.5). In addition, effects were noted at much lower dietary levels in offspring of treated rats (exposed during gestation and prior to weaning): significant increases in liver weight were noted in weanling rats after maternal exposure to only 5 ppm Aroclor 1260 and only 1 ppm Aroclor 1254 (179: Table III.9.2).

Increase in liver weight has been noted as an effect of PCBs in many other species (188, 193, 129, 210, 212, 214, 216), and is also a toxic affect of PCDFs (129, 71).

Table III.10.1 (ref. 219)

EFFECT OF PCB TREATMENT ON FINAL LIVER WEIGHTS AND ON LIVER:BODY WEIGHT RATIO IN MALE OSBORNE-MENDEL RATS[a]

Aroclor No.[b]	Dose (ppm)	Final body weight (g)	Liver weight (g)	Liver:body weight ratio
1242	500	273 ± 20	12.98 ± 1.03	0.047 ± 0.002[c]
	50	297 ± 31	9.93 ± 1.40	0.033 ± 0.002
	5	295 ± 33	9.19 ± 1.16	0.031 ± 0.001
	0.5	290 ± 7	8.71 ± 0.67	0.030 ± 0.001
	0	287 ± 18	8.69 ± 0.69	0.030 ± 0.001
1248	500	307 ± 25	16.90 ± 2.31	0.055 ± 0.005[c]
	50	329 ± 16	11.59 ± 1.16	0.035 ± 0.003[d]
	5	290 ± 30	8.70 ± 1.64	0.030 ± 0.003
	0.5	274 ± 13	8.53 ± 0.95	0.031 ± 0.002
	0	318 ± 26	9.93 ± 1.24	0.031 ± 0.002
1254	500	240 ± 33	15.14 ± 2.53	0.063 ± 0.003[c]
	50	255 ± 24	9.43 ± 1.25	0.038 ± 0.003[c]
	5	259 ± 20	7.87 ± 0.68	0.030 ± 0.002
	0.5	262 ± 15	8.38 ± 1.23	0.032 ± 0.004
	0	272 ± 22	8.02 ± 0.89	0.029 ± 0.001
1260	500	267 ± 22	15.90 ± 2.56	0.060 ± 0.009[c]
	50	285 ± 15	11.96 ± 1.54	0.042 ± 0.004[c]
	5	278 ± 22	9.11 ± 0.85	0.033 ± 0.003
	0.5	259 ± 23	8.21 ± 0.65	0.031 ± 0.002
	0	286 ± 21	8.65 ± 0.98	0.030 ± 0.002

[a] Results are given as the mean of 6 rats ±SD.

[b] Polychlorinated biphenyl compounds containing 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.

[c] Significantly different from control group at $P \leq 0.001$ as judged by the student t test.

[d] $P < 0.05$.

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III.10.2 Proliferation of smooth endoplasmic reticulum.

The initial increase in liver weight is due mainly to proliferation of smooth surfaced membranes of the endoplasmic reticulum, a network of interconnected channels present in the cytoplasm of most animal cells. The endoplasmic reticulum contains complexes of enzymes which play a role in metabolizing foreign substances such as drugs or environmental pollutants. These foreign substances are usually transformed into substances which can be more easily excreted by the body. A control step in metabolism of most foreign agents involves an oxidation reaction mediated by enzyme complexes (220). When liver samples are homogenized, the endoplasmic reticulum is broken up into particles referred to as microsomes and the enzymes are often referred to as microsomal enzymes. The liver microsomal enzymes responsible for metabolizing substances not normally expected to be present in the human body are sometimes referred to as drug-metabolizing enzymes. Norback and Allen (221) observed a proliferation of smooth endoplasmic reticulum (SER) in rats fed PCBs for one to five weeks. Similar proliferation of SER has been observed in mouse and monkey livers (197, 210).

The proliferation of SER is accompanied by increased activity of the microsomal enzymes responsible for metabolizing drugs and other foreign substances. Related observable changes in liver include increase in protein content and cytochrome P-450* content of microsomes.

* Cytochrome P-450 -- a microsomal hemoprotein implicated as the terminal oxidase in hepatic enzyme systems.

The duration and intensity of action by many drugs, carcinogens and other environmental chemicals which enter the body depends to a large extent on the rates at which they are metabolized by the cytochrome P-450 containing mixed function oxidase system in liver cells (222). Alvares et al. (223) found that Aroclor 1254 treatment (25 mg/kg per day for 6 days) nearly tripled cytochrome P-450 concentrations in rat liver. Litterst et al. (219) observed a significant increase in microsomal cytochrome P-450 content in rats fed 50 and 500 ppm Aroclors 1242, 1248, 1254, and 1260 for four weeks. In addition, a P-450 increase was apparent at 5 ppm of Aroclors 1248, 1254 and 1260 (Table III.10.2). The increase in cytochrome P-450 content appears to be dose-related and to increase somewhat with increasing chlorine content.

III.10.3 Microsomal enzyme induction

Disruptions in normal enzyme activity have been observed even at dose levels which had no effect on liver weight or on liver-to-body weight/ratios (219). Several hepatic enzyme systems are affected at oral doses as low as 5 ppm (224) or even 0.5 ppm (219): "no effect levels" have not been found (Table III.10.3). A chronic exposure to low dose levels of PCB is likely to exact an effect on enzyme activity in human liver.

The ability of PCBs to induce enzymes was first shown by Risebrough et al. (225). Allen and Abrahamson (188) found that PCBs caused overall increase in enzyme activity of rat livers which varied from approximately 2 to 10 fold when expressed in relation to the

Table III.10.2 (from ref. 219)

EFFECT OF PCB TREATMENT ON LIVER MICROSOMAL PROTEIN AND CYTOCHROME P-450 CONTENT OF RATS[a]

Aroclor No.[b]	Dose (ppm)	Protein[c]	P-450[d]
1242	500	51 ± 2	1.26 ± 0.09[e]
	50	43 ± 4	0.69 ± 0.05
	5	45 ± 4	0.42 ± 0.03
	0.5	40 ± 3	0.51 ± 0.03
	0	45 ± 5	0.46 ± 0.04
1248	500	84 ± 2[e]	1.29 ± 0.11[e]
	50	54 ± 4	0.71 ± 0.04[e]
	5	54 ± 5	0.46 ± 0.02
	0.5	51 ± 4	0.59 ± 0.04
	0	66 ± 7	0.37 ± 0.05
1254	500	60 ± 3[e]	1.42 ± 0.12
	50	60 ± 4	0.88 ± 0.06
	5	45 ± 2	0.71 ± 0.04
	0.5	45 ± 2	0.61 ± 0.04
	0	40 ± 3	—
1260	500	100 ± 8[e]	1.24 ± 0.09[e]
	50	73 ± 4[e]	1.20 ± 0.10[e]
	5	60 ± 5	0.71 ± 0.04
	0.5	53 ± 3	0.54 ± 0.03
	0	47 ± 3	0.57 ± 0.02

[a] Values are the results ±SD of duplicate determinations conducted on a microsomal pellet from six pooled livers.
[b] PCB compounds containing from 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.
[c] Expressed as mg microsomal protein/g liver.
[d] Expressed as nmoles of cytochrome P-450/g of microsomal protein.
[e] Significantly different from control values at $P < 0.05$ as judged by the student t test.

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Table III.10.3 (from ref. 219)

EFFECT OF PCB TREATMENT ON DEMETHYLATION ACTIVITIES IN HEPATIC MICROSOMES OF RATS[a]

Aroclor No.[b]	Dose (ppm)	Substrate: Aminopyrine Units[c]	Aminopyrine % of control	Codeine Units[c]	Codeine % of control	Ethylmorphine Units[c]	Ethylmorphine % of control
1242	500	14.2 ± 0.4[d]	240	22.4 ± 0.3[d]	245	15.9 ± 0.1[d]	219
	50	9.6 ± 0.1[d]	163	15.1 ± 0.4[d]	165	11.2 ± 0.3[d]	155
	5	7.6 ± 0.4	129	11.6 ± 0.2	127	8.7 ± 0.2	121
	0.5	9.3 ± 0.2[e]	108	9.1 ± 0.2	99	7.8 ± 0.1	108
	0	5.9 ± 0.2	100	9.1 ± 0.1	100	7.2 ± 0.3	100
1248	500	17.4 ± 0.5[d]	272	24.8 ± 0.4[d]	258	17.6 ± 0.5[d]	243
	50	10.4 ± 0.4[d]	163	16.5 ± 0.4[d]	171	12.1 ± 0.5[d]	166
	5	7.6 ± 0.3	120	11.5 ± 0.3[e]	119	9.6 ± 0.4[e]	133
	0.5	5.1 ± 0.2[e]	79	8.5 ± 0.3[e]	88	7.4 ± 0.3	102
	0	6.4 ± 0.4	100	9.6 ± 0.1	100	7.2 ± 0.1	100
1254	500	19.3 ± 0.2[d]	336	25.3 ± 0.3[d]	264	17.9 ± 0.5[d]	236
	50	16.6 ± 0.2[d]	289	24.4 ± 0.5[d]	253	17.9 ± 0.4[d]	236
	5	8.9 ± 0.3[d]	155	13.2 ± 0.3[d]	138	11.1 ± 0.2[d]	146
	0.5	7.2 ± 0.2[e]	127	12.2 ± 0.2[d]	127	9.6 ± 0.2[e]	127
	0	5.8 ± 0.2	100	9.6 ± 0.2	100	7.6 ± 0.3	100
1260	500	22.2 ± 0.2[d]	274	28.5 ± 0.5[d]	197	17.4 ± 0.3[d]	162
	50	18.6 ± 0.3[d]	229	26.6 ± 0.3[d]	183	18.0 ± 0.2[d]	167
	5	13.0 ± 0.3[d]	160	20.7 ± 0.3[d]	143	15.9 ± 0.2[d]	148
	0.5	9.6 ± 0.3[d]	118	14.9 ± 0.3	103	12.4 ± 0.3[e]	116
	0	8.1 ± 0.1	100	14.5 ± 0.1	100	10.7 ± 0.2	100

[a] Each value is the mean ±SD of 3 replicates.
[b] Polychlorinated biphenyl compounds containing 42% (1242) to 60% (1260) by weight of chlorine, fed in the diet for 4 wk.
[c] Expressed as μmoles of product formed/g liver/30 min.
[d] Significantly different from control group at $P < 0.01$ as judged by the student t test.
[e] $P < 0.05$.

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total weight of the liver. Since PCBs are relatively resistant to metabolic breakdown, they persist as enzyme inducers. As the enzymes are continuously induced, hormones and perhaps other compounds may be metabolized at rates faster than normal over the whole life span of the organism. Increased metabolism of several hormones in birds and mammals has been demonstrated (225-227).

After a single oral dose of 50 mg/kg body weight Aroclor 1254 administered via stomach tube, microsomal enzyme activities increased until 24 hours after treatment and then either remained elevated or declined. Enzyme activities were still significantly increased over control values 48 hours after the single administration (228). Young rats are more susceptible to the enzyme inducing effects than adult rats (229).

With continuous exposure to high dietary levels of PCBs, a decrease is eventually observed in SER proliferation and concentric membrane arrays pervade the cytoplasmic reticulum. The induced levels of microsomal enzyme activity persist (221).

After termination of exposure, the enzyme inducing effect of PCBs appears to be reversible. Litterst and Van Loon (228) maintained rats on a diet of 50 ppm PCB for seven days. Discontinuation of PCB treatment resulted in a slow decay of the induced enzyme activity to approximately control levels after ten days.

Increased activities of the drug metabolizing enzymes aniline hydroxylase and aminopyrene n-demethylase were observed in pregnant rabbits exposed to single doses of 10 mg/kg Aroclor 1254 (217).

Carboxylesterase activity and the ability to degrade parathion to p-nitrophenol were significantly increased by the same doses. The effects of Aroclor 1221 were smaller than those of Aroclor 1254 at the same doses, but both Aroclors affected vitamin A storage after a single dose of 1 mg/kg (217).

Application of very small amounts of PCBs to the skin of experimental animals can cause a marked increase in mixed function oxidase activity (220).

A significant increase in activity of demethylating, reducing and hydroxylating microsomal enzymes was observed in livers of rats fed 150 μmoles per kg of food for 30 days. PCBs at this dose produced a significantly greater increase in activity than did DDT on all but the demethylating reaction where the effect was equal (228).

Specific enzyme activity, expressed per unit weight of microsomal protein or per unit weight of liver may increase or decrease under the influence of PCBs. However, when these data are expressed in relation to the total liver, a decided increase has been observed in activity of all enzymes assayed (188). PCB exposure can cause changes in activity of specific enzyme pathways in liver microsomes. The following alterations in enzyme activity have been observed:

- o Nitroreductase -- Specific activity increased during the first week under the influence of Aroclors 1248 and 1254. Activity returned to near normal values during the second week and continued to decrease through the sixth week. In rats fed Aroclor 1262, a much greater increase in activity persisted (188).

  Litterst et al. (219) observed a striking increase in nitroreduction of p-nitrobenzoic acid. The effect was dose-

related. The activity of nitroreductase in rats fed 500 ppm of Aroclor 1260 was 10 times the control values while all 4 Aroclors (1242, 1248, 1254, and 1260) at 0.5 ppm caused a 50 to 70 percent increase in activity (Figure III.10.1). The nitroreductase induction effect increased with increasing chlorine content (219). Nitroreductase activity levels continued to increase during four weeks of treatment with PCBs (228).

- Demethylase -- PCBs induced activity of demethylases in rat liver microsomes. The increase in amounts of product formed with increased PCB concentrations suggests that a dose-response relationship exists between any one specific Aroclor and this enzyme. Litterst et al. (219) observed that demethylase activity reached a peak at 54 percent chlorine content and then decreased in amplitude with Aroclor 1260 (Table III.10.3). Chen and DuBois (229), however, observed an increase in demethylase activity with a degree of chlorination for Aroclors 1221, 1254, and 1260.

  Even at the highest dose of all the Aroclors tested, demethylase activities do not exceed a level of 2 to 3 times the control values (219). In PCB-treated rats, demethylase activity reached the maximum level of induction within seven days (228).

  Vaino (230) observed a 16-fold increase in p-nitroanisole O-demethylase activity after treating rats for 6 days with daily injections of Clophen A50 (15 mg/kg).

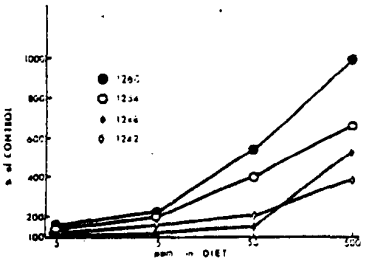
  Allen and Abrahamson (188) observed an increase in specific activity of rat liver demethylase throughout the 6-week PCB feeding period.

- Deethylase -- Wilson and Hansen (231) observed a marked increase in deethylation of p-nitrophenetole in microsomal preparations from sheep after feeding for 15 weeks with 20 ppm Aroclors 1242 or 1254 (Table III.10.4).

- Glucose-6-phosphatase -- Specific activity in rat liver decreased continuously for six weeks (188). Litterst et al. (219) also observed a decrease in specific activity of glucose-6-phosphatase in livers of PCB-dosed rats: the effect was most marked for Aroclor 1242 and was significant at the lowest dose tested (0.5 ppm for 4 weeks).

- Hydroxylase -- Although overall increase of hydroxylase activity has been consistently observed on administration of PCBs, patterns of change in specific hydroxylase activity observed by different investigators have varied.

Figure III.10.1 (from ref. 219)



Dose-response relationship of 4 Aroclors on nitroreduction of *p*-nitrobenzoic acid in rat liver microsomes. Each point represents the mean of 3 replicates. The SD values were all less than 5% of their respective means.

Table III.10.4 (from ref. 231)

SPECIFIC ACTIVITY OF *p*-NITROPHENETOLE *O*-DEETHYLATION COMPARED TO THE SLOPE OF LINEWEAVER-BURKE KINETIC PLOTS AT LOW (0.0125–0.15 mM) AND HIGH (0.15–2 mM) SUBSTRATE CONCENTRATIONS

Treatment	Specific activity[a]			Slope	
	0.05 mM[b]	0.5 mM	1 mM	0.15–2 mM	0.0125–0.15 mM
Normal control	2.67	6.98	7.48	0.0233	0.0035
Normal 1242	4.07	6.66	7.61	0.0103	0.0022
Normal 1254	5.67	9.48	11.22	0.0074	0.0024
Low-protein control	5.44	8.40	10.36	0.0177	0.0025
Low-protein 1242	9.26	13.07	14.94	0.0094	0.0022
Low-protein 1254	13.72	16.82	18.35	0.0030	0.0023
High-fat control	10.70	16.94	17.31	0.0030	0.0023
High-fat 1242	4.55	7.36	7.30	0.0089	0.0023
High-fat 1254	8.42	12.60	14.08	0.0092	0.0034

[a] nmol/min/mg protein.
[b] Substrate concentration.

Vaino (230) observed a 7.5-fold increase in hepatic aryl hydrocarbon hydroxylase activity after treating rats for 6 days with consecutive daily injections of 15 mg/kg Clophan A50. A slight enhancement in the overall hydroxylation reaction was already observable 24 hours after a single intraperitoneal injection.

Allen and Abrahamson (188) observed a continuous decrease in specific activity of aromatic hydroxylase in rat liver for six weeks: this anomalous result is perhaps due to the very high dose used, which caused overt liver damage and ultimately death.

Litterst and Van Loon (228) observed two peaks in hydroxylase activity after PCB administration to rats. One peak occured 7 days after treatment and the other peak occured 28 days after treatment. Activity was slightly lower after 14 days than it had been after 7 days. Litterst and Van Loon used ^{14}C-pentobarbital as their substrate.

Alvares et al. observed an 11-fold increase in activity of benzo(e)-pyrene hydroxylase after application of microscope immersion oil containing PCBs to rat skin (222).

- o Esterase -- Specific activity decreased continuously for rats on Aroclor 1248 and 1254 diets. With Aroclor 1262 there was a slight increase the first week, then specific activity leveled off (188).

By increasing activity of drug-metabolizing enzymes, PCBs can reduce the pharmacological effect of certain drugs. One parameter used to measure enzyme induction is reduction of hexobarbital or pentobarbital induced sleeping time (220). After treatment with PCBs, the rate at which rats metabolize the barbiturates to non-narcotic substances is increased. Decrease in pentobarbital sleeping time following dietary administration of several PCB isomers was demonstrated in rats (232, 233), mice (234) and rabbits (235). Diminished pharmacological actions of zoxazolamine and hexobarbital have also been observed (236).

The various effects of PCBs on liver microsomal enzymes can be blocked by the prior administration of actinomycin D (5). Actinomycin D acts as an inhibitor of DNA-dependent RNA synthesis. Alvares et al. (223) interpreted the inhibitory effect of Actinomycin D on PCB-induced increases in enzyme activity as support of the view that PCBs enhance the synthesis of a distinct microsomal hemoprotein not present in liver of untreated rats.

There are substances other than PCBs which are also known to increase the activity of drug metabolizing enzymes in the liver microsomes. Litterst and Van Loon (228) compared the enzyme-inducing effect of Aroclor 1254 with the effects of DDT and phenobarbital. Rats were fed 5, 15, or 150 u moles per kg of food for 30 days. They concluded that at 150 u moles per kg food, PCBs were far more effective enzyme inducers than phenobarbital and at least as effective as DDT.

Two types of inducers of drug-metabolizing enzymes of the liver have been reported. One group, which includes phenobarbital, enhances the metabolism of a large variety of substrates. The other group, which includes such polycyclic aromatic hydrocarbons (PAH) as benzo(a)-pyrene and 3-methylcholanthrene, stimulates the metabolism of only a few compounds. The effects of these two groups of inducers on cytochrome P-450 differ. Cytochrome P-450 is a microsomal hemoprotein which has been implicated as the terminal oxidase in the hepatic enzyme systems. Treatment of rats with inducers from either group increases concentration of cytochrome P-450, but

the PAH also induce formation of cytochrome P-448. Cytochrome P-448 differs from cytochrome P-450 in spectral properties and catalytic activities (223). Polychlorinated biphenyls appear to be a new type of hepatic enzyme inducer, sharing the properties of both groups. PCBs cause the formation of cytochrome P-448 (like the PAH group) while eliciting the more general enzyme induction response of the phenobarbital group.

Data of Vaino (230) support the hypothesis that PCBs form a new type of inducer group enhancing drug biotransformation in liver microsomes. PCBs were observed to produce a cytochrome P-448 with catalytic properties differing from those of P-448 induced by PAH (223, 230). PCBs were also able to increase the NADPH cytochrome reductase activity which is uninfluenced by PAH. Alvares et al. (222) also reported induction of NADPH cytochrome c reductase in addition to cytochrome P-448 by PCBs.

Barbiturates and PAH both stimulate the UDP glucuronosyltransferase activity of liver microsomes. The enhancement of UDP glucuronosyltransferase activity caused by Clophen A50 was considerably greater than obtained by any of the previously known inducers (230).

Wilson and Hanson (231) have also given evidence from the kinetics of O-deethylating reactions in sheep microsomal preparations that PCBs stimulate two distinct types of enzyme system.

Alvares and Siekavitz (237) have shown by means of gel electrophoresis that PCBs induce three distinct subunits of cytochrome P-450.

III.10.4. Structure-activity relationships.

A number of recent studies have started to elucidate structure-activity relationships in enzyme induction by chlorobiphenyls (146, 224, 238-242). The results of some of these studies are summarized in Figures III.10.2 - III.10.6 (from ref. 242).

Figure III.10.3 summarizes the influence of the various agents tested on the hepatic mixed function oxidases aniline hydroxylase (A), p-nitroanisole O-demethylase (B), and aminopyrine N-demethylase (C). The most responsive enzymes were those closely associated with the smooth endoplasmic reticulum. The results in vitro reflected the observations of altered sleeping times in vivo (Figure III.10.2). One can see that, even with this limited number of pure chlorobiphenyls, not all caused effects of the same magnitude. To summarize the results of this study, treatment with biphenyl caused slight induction while 4-chlorobiphenyl did not. The mixed function oxidases were markedly induced by pure hexa- and octa-chlorobiphenyls and also by di- and tetra-chlorobiphenyls with chlorines substituted at the 4-positions of the rings. Considering the dichloro-isomers, when the 4- and 4'-positions were occupied, there was a much greater inductive effect for all of the enzyme activities than was observed with the 2,2'- and 2,4'-isomers. The same positional phenomenon was observed for the two tetra-chlorobiphenyls studied, the induction caused by 2,4,2',4'-tetra-chlorobiphenyl being much greater than that observed with the 2,5,2',5'-isomer. The results obtained for the higher chlorinated analogs suggested that the positions of the

III. TOXIC EFFECTS

III. 1. Introduction

Toxicological aspects of PCBs have been reviewed in refs. 1, 2 and 5, and in a number of papers presented at the National Conference on Polychlorinated Biphenyls in November 1975 (9, 16, 29, 35, 67-75). The following summary is concerned especially with three aspects of the toxicology of PCBs:

1. Definition of the toxic effects which have been observed reliably at low levels of exposure to PCBs; these are of greatest importance in establishing criteria.

2. Review of the differences in toxicity between the various commercial mixtures, which reflect differences in toxicity among the individual chlorobiphenyls.

3. Elucidation of the role of PCDFs in the observed toxicity of commercial mixtures or environmental residues.

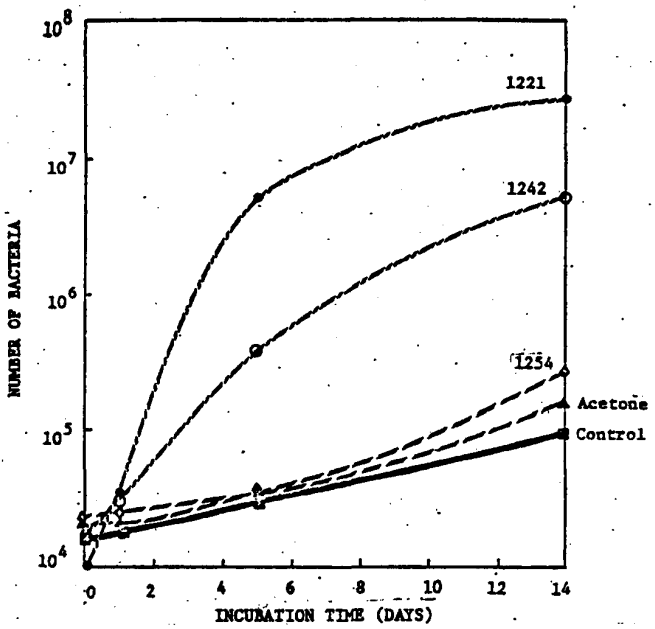
III. 2. Effects on microbial systems.

The effects of PCBs on micro-organisms range from stimulus to inhibition of growth, depending upon the degree of chlorination, the type of organism, and other environmental factors. When Aroclors 1221, 1242, and 1254 were added to bacteria from lake water (including Achromobacter sp. and Pseudomonas sp.) in 1 percent glucose medium, no inhibition of growth occurred at concentrations up to 0.1 percent Aroclor. In fact, there was a slight stimulation by Aroclors 1221 and 1242 (76). Lake water samples incubated with Aroclors as the carbon and energy sources illustrated that bacteria could use 0.05 percent Aroclors 1221 and 1242

for growth support (see Figure III.2.1) but not Aroclor 1254 (76). The ability of the bacteria to utilize PCBs decreased with an increase in percent of chlorination.

When natural waters were enriched by the addition of 0.1 ppm Aroclor 1260, bacterial counts yielded increasing numbers of viable bacteria (41). No adverse effects were noted, with growth of the treated cells exceeding that of the control (see Table III.2.1). In spite of the slight growth enhancement, no metabolites were detected and unaltered parent compounds were recovered.

Isolates of estuarine bacteria (including Flavobacterium, Bacillus, Corynebacterium, Pseudomonas, Achromobacter, Micrococcus, and Serratia) were grown on agar with Aroclor 1016 and 1242 saturated paper discs (77). Of 85 tested cultures, 26 were inhibited to varying extents by 0.5 mg (5 mg in 0.1 ml acetone) of either Aroclor. Cultures sensitive to Aroclor 1242 were also sensitive to Aroclor 1016, and 65 percent of the cultures were still sensitive at 0.1 mg. Concentrations of 0.1 mg Aroclor 1016 inhibited 58 percent of the cultures sensitive at 0.5 mg. Four representative cultures of the sensitive bacteria (two gram-positive and two gram-negative) were tested for growth inhibition in liquid culture with 10 $\mu g/ml$ (10 ppm) of Aroclor 1242. The extended lag times suggest an adaptation mechanism (see Figure III.2.2) and possibly extended time (greater than the 24 hours used) on agar would produce similar results. Previous reports indicate that gram-positive bacterial growth was inhibited by organochlorine insecticides (chlordane) and gram-negative bacteria were unaffected (77). The bacteria sensitive to Aroclor 1254 were both



The time/growth curve of bacteria in 0.05% Aroclor 1221, Aroclor 1242, and Aroclor 1254 as carbon and energy sources.

Figure III.2.1 (from ref. 76)

Table III.2.1 (from ref. 41)

The fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from the Fraser River, held in the laboratory for 12 weeks at 7°C

Time, weeks	Compound	Bacteria /ml	Recovery, % Water	Glass wool
0	α-Chlordane	1.8×10^{2}	98.7	0*
6	α-Chlordane	1.3×10^{4}	59.8	9.5
6	α-Chlordane	2.3×10^{4}	59.7	17.7
12	α-Chlordane	4.2×10^{5}	41.7	7.6
12	α-Chlordane	8.8×10^{4}	44.1	6.3
0	γ-Chlordane	1.8×10^{2}	90.6	0
6	γ-Chlordane	2.2×10^{4}	53.0	18.6
6	γ-Chlordane	2.5×10^{4}	55.6	6.4
12	γ-Chlordane	2.6×10^{5}	37.1	9.4
12	γ-Chlordane	7.1×10^{4}	38.1	3.4
0	Lindane	1.8×10^{2}	101.0	0
6	Lindane	1.2×10^{4}	99.7	0
6	Lindane	1.4×10^{4}	101.5	0
12	Lindane	5.3×10^{5}	96.7	0
12	Lindane	5.3×10^{4}	97.2	0
0	DDT	2.0×10^{2}	95.6	0
6	DDT	1.8×10^{4}	67.4	5.6
6	DDT	2.2×10^{4}	71.3	3.2
12	DDT	4.8×10^{4}	69.7	7.2
12	DDT	3.4×10^{4}	68.7	3.9
0	PCB[a]	1.9×10^{2}	95.6	0
6	PCB[a]	5.2×10^{4}	60.1	1.8
6	PCB[a]	2.7×10^{4}	60.9	4.5
12	PCB[a]	1.1×10^{5}	64.1	1.2
12	PCB[a]	—	74.7	5.5
0	Acetone only	2.2×10^{2}	0	0
6	Acetone only	9.3×10^{3}	0	0
12	Acetone only	4.6×10^{4}	0	0
0	Control	2.3×10^{2}	0	0
6	Control	7.1×10^{3}	0	0
12	Control	4.5×10^{3}	0	0

*No residue detected. [a] 0.1 ppm Aroclor 1260.

The fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from the Nicbatsk'l River, held in the laboratory for 12 weeks at 16°C

Time, weeks	Compound	Bacteria /ml	Recovery, % Water	Glass wool
0	α-Chlordane	1.5×10^{4}	92.0	0*
6	α-Chlordane	—	50.2	0.8
6	α-Chlordane	—	51.1	1.5
12	α-Chlordane	1.1×10^{5}	20.5	1.2
12	α-Chlordane	5.7×10^{5}	16.6	0.8
0	γ-Chlordane	6.4×10^{3}	100.8	0
6	γ-Chlordane	—	34.0	0.2
6	γ-Chlordane	—	24.7	1.3
12	γ-Chlordane	1.1×10^{5}	2.3	0.5
12	γ-Chlordane	5.4×10^{4}	13.9	1.5
0	Lindane	8.5×10^{3}	98.4	0
6	Lindane	—	90.5	0
6	Lindane	—	88.5	0
12	Lindane	1.2×10^{5}	79.0	0
12	Lindane	4.0×10^{5}	76.0	0
0	DDT	1.5×10^{4}	96.3	0
6	DDT	—	34.4	2.2
6	DDT	—	43.7	3.4
12	DDT	9.9×10^{4}	30.1	1.1
12	DDT	1.4×10^{5}	23.6	3.5
0	PCB[a]	2.4×10^{4}	96.2	0
6	PCB[a]	—	73.8	1.4
6	PCB[a]	—	74.5	1.6
12	PCB[a]	7.5×10^{5}	33.0	1.1
12	PCB[a]	4.4×10^{5}	56.7	1.1
0	Acetone only	1.8×10^{4}	0	0
6	Acetone only	—	0	0
12	Acetone only	2.6×10^{4}	0	0
0	Control	4.9×10^{4}	0	0
6	Control	—	0	0
12	Control	1.4×10^{4}	0	0

*No residue detected. [a] 0.1 ppm Aroclor 1260.

The fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from Georgia Strait, held in the laboratory for 12 weeks at 9°C

Time, weeks	Compound	Bacteria /ml	Recovery, % Water	Glass wool
0	α-Chlordane	1.0×10^{2}	91.3	0*
6	α-Chlordane	1.1×10^{3}	41.9	0.8
6	α-Chlordane	8.9×10^{3}	30.3	1.0
12	α-Chlordane	3.0×10^{3}	50.7	0.6
12	α-Chlordane	1.3×10^{5}	53.2	0.6
0	γ-Chlordane	1.1×10^{2}	94.4	0
6	γ-Chlordane	9.3×10^{4}	31.9	21.5
6	γ-Chlordane	—	24.9	9.5
12	γ-Chlordane	4.5×10^{4}	28.4	9.6
12	γ-Chlordane	1.3×10^{4}	35.6	4.3
0	Lindane	8.6×10^{2}	99.9	0
6	Lindane	1.4×10^{6}	91.5	0
6	Lindane	6.0×10^{4}	96.0	0
12	Lindane	1.3×10^{4}	99.2	0
12	Lindane	1.1×10^{4}	90.5	0
0	DDT	8.0×10^{2}	90.8	0
6	DDT	5.8×10^{4}	65.8	3.2
6	DDT	7.2×10^{4}	49.3	17.2
12	DDT	1.1×10^{4}	53.0	6.0
12	DDT	1.9×10^{3}	36.6	10.7
0	PCB[a]	7.8×10^{2}	93.5	0
6	PCB[a]	9.8×10^{4}	70.7	1.3
6	PCB[a]	6.5×10^{4}	40.7	6.2
12	PCB[a]	3.5×10^{3}	37.7	2.4
12	PCB[a]	1.1×10^{3}	38.7	0.8
0	Acetone only	1.1×10^{3}	0	0
6	Acetone only	3.4×10^{4}	0	0
12	Acetone only	4.5×10^{3}	0	0
0	Control	8.0×10^{2}	0	0
6	Control	2.4×10^{3}	0	0
12	Control	5.1×10^{3}	0	0

*No residue detected. [a] 0.1 ppm Aroclor 1260.

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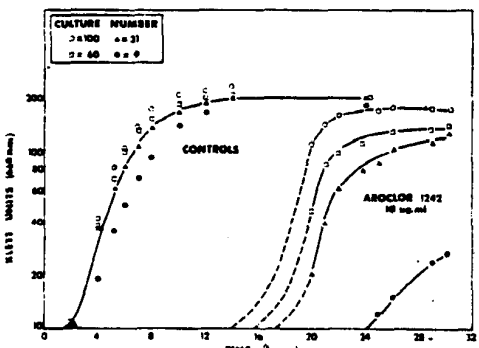
gram-positive and negative, with slightly more (61 percent) of the sensitive bacteria gram-positive. When PCB-sensitive and non-sensitive strains were compared, the biochemical activities of test bacteria fell into two different groups. Amylase was produced by 76 percent and gelatinase by 86 percent of the sensitive strains, whereas the nonsensitive strains showed only 33 percent and 42 percent activities, respectively. No conclusions were drawn by Bourquin and Cassidy (77) as to the possible significance of the prevalence of PCB-sensitivity among amylase and gelatinase producers. However, they suggested further investigation to determine any relationships to total nutrient catabolism. In another recent paper, Bourquin *et al.* (78) again reported little difference in activity between Aroclors 1242 and 1016. Results for 25 of the 100 cultures tested are shown in Table III.2.2.

Using *Escherichia coli*, a common human intestinal aerobe, Keil *et al.* (79) found that Aroclor 1242 did not inhibit growth at concentrations up to 1,000 ppm. Aroclor 1242 at a concentration of 0.1 ug/ml^{-1} (0.1 ppm), and possibly at 0.01 ug/ml^{-1} (0.01 ppm), stimulated *E. coli* growth (see Table III.2.3).

In a separate experiment using *E. coli* and *Bacteriodes fragilis* (an intestinal anaerobe), Greer *et al.* (80) found no detectable effect on growth using 0.01 ppm (0.01 mg/l) and 0.1 ppm (0.1 mg/l) Aroclor 1242. However, there was an apparently dose-related depression of lipid synthesis in *B. fragilis* only (see Table III.2.4).

The effect of 2,2'-dichlorobiphenyl on microbial predators of *E. coli* was found to decrease kill rates at lower concentrations (81).



Four sensitive bacteria were tested for growth in one-half strength 2216 Marine Broth containing 10 ug of Aroclor 1242 per ml. Symbols: (○) Achromobacter sp., (●) Bacillus sp., (▲) Corynebacterium sp., and (□) Pseudomonas sp. Controls contain one-half strength 2216 Marine Broth only; all salinities were 20‰.

Figure III.2.2 (from ref. 77)

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Table III.2.2 (from ref. 78)

Inhibition of growth of estuarine bacteria on nutrient seawater medium by PCB's

GBERL Culture No.	Gram Reaction & Morphology	Genus	Aroclor® 1242 (mg)			Aroclor® 1016 (mg)		
			0.1	0.25	0.5	0.1	0.25	0.5
5	+ ROD	Unknown	++	++	+++	++	++	+++
21	− ROD	Unknown	++	++	+++	++	++	+++
38	− ROD	*Flavobacterium* sp.	++	++	++	+	+++	+++
39	− COCCOID	Unknown	++	+++	+++	++	+++	+++
53	− ROD	Unknown	++	+++	+++	+++	+++	+++
54	+ ROD	*Bacillus* sp.	+++	+++	+++	+	+++	+++
7	+ ROD	*Bacillus* sp.	+	+	+	x	+	++
9	+ ROD	*Bacillus* sp.	+	++	+++	+	++	+++
31	− ROD	Unknown	+	+	+++	+	+	+++
60	+ ROD	*Bacillus* sp.	+	+	+	++	++	++
86	− ROD	*Flavobacterium* sp.	+	+	+	x	+	+
100	− ROD	*Pseudomonas* sp.	+	++	++	+	++	++
8	+ ROD	*Corynebacterium* sp.	x	+	++	x	+	++
11	− ROD	*Achromobacter* sp.	x	+	++	x	+	++
42	+ COCCUS	*Micrococcus* sp.	x	+	++	x	+	++
44	+ COCCUS	*Micrococcus* sp.	x	+	+	−	x	+
93	+ ROD	Unknown	x	+	+	−	x	+
43	+ COCCUS	*Micrococcus* sp.	−	+	++	−	+	++
5	− COCCOID	*Serratia* sp.	−	−	++	−	−	++
13	− ROD	*Achromobacter* sp.	−	−	++	−	−	++
28	− ROD	*Achromobacter* sp.	−	−	++	−	−	++
32	+ ROD	*Corynebacterium* sp.	−	+	+	−	x	+
41	− COCCOID	Unknown	−	−	++	−	−	++
67	− ROD	*Achromobacter* sp.	−	+	+	−	−	+
69	− ROD	Unknown	−	−	++	−	−	++

Degree of sensitivity: +++ (18-20 mm zone), ++ (16-18 mm), + (14-16 mm), x (slightly), − (not sensitive).

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Table III.2.3 (from ref. 79)

LYOPHILIZED WEIGHTS AND TOXICANT UPTAKE BY ESCHERICHIA COLI CULTURES EXPOSED TO PCB

Treatment	Mean Harvest Weights (mg)	Mean Residue ($\mu g\ g^{-1}$)
Bacterial Series I		
PCB 0.1 $\mu g\ ml^{-1}$	86.0	13.6
PCB 0.01 $\mu g\ ml^{-1}$	90.4	4.4
Control	44.5	0
Acetone control	27.2	1.2
Uridine control	41.6	0
LSD_{05}*	13.6	10.8
Bacterial Series II		
PCB 0.1 $\mu g\ ml^{-1}$	186.4	
PCB 0.01 $\mu g\ ml^{-1}$	153.2	
Control	160.8	
Acetone control	133.6	
LSD_{05}*	23.6	

* LSD_{05} = Least significant at 95 percent probability level calculated from one way analysis of variance.

Table III.2.4 (from ref. 80)

HARVEST WEIGHTS, TOXICANT RESIDUE, AND LIPID YIELDS OF BACTERIODES FRAGILIS CULTURES TREATED WITH PCB

Treatment	No. Replicates	Mean Harvest Weight (mg)	Mean Toxicant Residues (μg)	Mean Lipid Yields (μg/mg dry wt)
.01 ppm PCB	4	137.4	N.D.*	23.2
.1 ppm PCB	2	210.4	N.D.*	16.2
Control	4	119.7	0	34.9
Acetone Control	4	109.9	0	35.7
LSD_{05}**	4/4	26.0		7.8
	2/4	31.8		9.5

* N.D. = Not Determined

** LSD = Least significant difference at 95 percent probability level calculated from one way analysis of variance.

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The predators (the dominant Spirillum spp. and a pseudomonad), were isolated from sea water using E. coli as the sole carbon source. Neither the predator nor E. coli viability and activity were affected by 10^{-3}M (200 ppm) 2,2'-dichlorobiphenyl. Table III.2.5 shows percentage kill of E. coli in the presence of varying predator populations and dichlorobiphenyl concentrations.

Walsh and Mitchell (81) noted that the lower dichlorobiphenyl concentrations (10^{-5}M) which generally decreased E. coli kill are similar to those used previously to inhibit chemotaxis in motile marine bacteria. The higher concentrations (10^{-3}M) which increased kill rates in the standing flasks for lower predator populations were similar to those Walsh and Mitchell observed to increase chemotaxis. The standing flasks probably contained a higher concentration of both microbes and chemicals at the surface (41). Thus, the changes in E. coli kill by microbial predators may be due to inhibition or excitation of the chemotactic response by dichlorobiphenyls.

PCBs may cause changes in the ecological role of microorganisms. The differing sensitivities of the bacteria to the PCBs present may cause changes in composition of community populations. For example, Escherichia coli appears relatively resistant to PCBs at the concentrations tested (79-81). Stimulation of E. coli growth occurred at lower concentrations (0.1 and 0.01 ppm) of Aroclor 1242 (79). Achromobacter sp. and Pseudomonas sp. also had growth slightly stimulated with 500 ppm Aroclors 1242 and 1221. Oloffs et al. (41) increased bacterial numbers in natural waters by the addition of 0.1 ppm Aroclor 1260. If, as Bourquin and Cassidy (77) reported, one-third of a natural

Table III.2.5 (from ref. 81)

Percentage kill of *E. coli* in 48 h by motile marine predators in the presence of chlorinated hydrocarbons

Initial predator population no. per ml.	No chlorinated hydrocarbons	2, 4-D		*o, o*-Dichlorobiphenyl	
		10^{-4} M	7×10^{-5} M	10^{-4} M	10^{-5} M
Standing	% Kill of *E. coli*				
10	0	7	31	0	31
10^{6}	6	3	32	17	35
10^{8}	30	10	29	13	39
10^{9}	72	26	41	41	47
Fully mixed					
10	20	23	25	18	27
10^{6}	54	29	41	31	44
10^{8}	61	32	43	30	43
10^{9}	71	77	44	55	51

The initial *E. coli* population was 10^{7} ml^{-1}. The data are corrected for kill observed in the absence of the predators.

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estuarine bacterial population is inhibited by chlorinated biphenyls and stimulation of some of the remaining two-thirds is possible (41,79), with no response by other members, a totally different bacterial community may be created. Although the concentrations of PCBs required to show effects on single species cultures are much higher than those occurring in natural waters, they are comparable with those reported from sediments and sewage sludge (2,10-12, 48-50). Moreover, as in the case of phytoplankton (Section III.3), effects on mixed-species cultures may take place at lower concentrations of PCBs.

Changes in community composition, coupled with possible increased (or decreased) chemotactic responses of predatory micro-organisms (81) may cause further disruption of normal carbon cycling and pollution control associated with microbes. It may be necessary, in light of the effects of PCBs, to change some of the criteria presently used to determine water pollution. For example, low counts of *E. coli* may be indicative of high PCB levels stimulating predators, not clean water.

Natural adaptive mechanisms of micro-organisms may compound or counteract the effects of PCBs. Baxter *et al.* (82) stressed the adaptation of *Nocardia* spp. grown on biphenyl. The ability to degrade PCBs and the rate of degradation appear dependent upon degree of chlorination as well as available precursors. Thus, microbial communities may undergo continual changes or cycles as bacteria, chemical components and the associated processes and dependents fluctuate.

III.3 Effects on Phytoplankton

Phytoplankton are the photosynthetic organisms which form

the basis for all marine and some fresh-water food-webs. A number of experiments have shown that PCBs interfere with photosynthetic mechanisms in certain species of phytoplankton at concentrations as low as 0.1 ppb (ug/l) or below (83-92). In most cases the effects measured were reductions in the uptake of radiolabelled carbon (supplied as bicarbonate), a measure of the rate of carbon fixation. In a recent assessment, Fisher (83) has suggested that the primary effects of PCBs are to reduce the rates of cell growth and cell division; the important consequences are likely to be changes in the species composition of marine phytoplankton communities, rather than a reduction in the total rate of photosynthesis by the community.

The radiocarbon uptake of two green algae, Scenedesmus quadricauda (fresh water) and Dunaliella tertiolecta (estuarine) was reduced by 60 percent and 35 percent, respectively, when they were exposed to Aroclor 1254 at 100 ppb (84). Effects on S. quadricauda were marked even at 0.1 ppb, the lowest concentration tested. (Table III.3.1). Inhibition was greater in Scenedesmus which possesses a cell wall, as opposed to the naked Dunaliella (see Table III.3.1). Since the cell wall appears not to prevent absorption, either the cell or chloroplast membrane would appear to be the selective barrier. There was no effect on radiocarbon uptake in either alga by DDT in concentrations up to 1 ppm. However, photoreduction of isolated chloroplasts was inhibited by both PCBs and DDT, suggesting that the cell membrane possesses an active transport site preferentially structured for PCB (84).

Table III.3.1 (from ref. 84)

INHIBITION OF CARBON-14 UPTAKE IN SCENEDESMUS AND DUNALIELLA BY CHLORINATED HYDROCARBONS

Concentration ppb	C^{14} Uptake, % Control		
	DDT	Aroclor 1242[a]	Aroclor 1242[b]
0.1	90.66	79.81	113.2
1	111.44	70.45	94.1
10	98.85	74.02	103.6
10^2	109.12	40.39	65.7
10^3	101.74	34.53	56.9

[a] Scenedesmus

[b] Dunaliella

Source: Luard, E.J. 1973. Sensitivity of Dunaliella and Scenedesmus (Chlorophyceae) to chlorinated hydrocarbons. Phycologia 12(1/2): 29-33.

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Exposure of the marine diatom Cylindrotheca closterium to 10 or 100 ppb Aroclor 1242 resulted in inhibition of growth and diminished levels of chlorophyll and RNA synthesis (Table III.3.2). Levels of DNA were not affected, implying selective action by the polychlorinated biphenyls on cell metabolism (85). The chlorophyll content per cell was similar in the treated and control cultures, suggesting that the primary effect of PCBs was to inhibit cell growth and division rather than to inhibit photosynthesis per se. A similar result was obtained by Fisher (83) using two other diatom species exposed to 10 ppb Aroclor 1254.

Sensitivity of two marine diatoms Thallassiosira pseudonana and Rhizosolenia setigera to Aroclor 1254 increased with decreasing temperature (Table III.3.3)(87). R. setigera had growth completely stopped for 48 hours at concentrations of 0.1 to 10 ppb Aroclor 1254, after which growth resumed with less inhibition at the higher temperature (87: Figure III.3.1). T. pseudonana was only sensitive at 10 ppb Aroclor 1254. The effects of temperature, Aroclor 1254, and the interaction between temperature and Aroclor 1254 were all significant ($P < 0.001$), indicating that sensitivity to PCBs was dependent on temperature. The increased inhibition at temperatures at which cell division rates were slowest illustrates the narrowing of a sensitive species' niche with environmental stress. Thus, species more vulnerable to stress would be less capable of successful competition for resources. If this were true, species adapted to stressful conditions, such as occur in estuarine environments with salinity changes, would be expected to withstand higher levels of PCBs. Supporting this hypothesis is the finding

Table III.3.2 (from ref. 85)

HARVEST WEIGHT, CELL COUNTS, NUCLEIC ACID LEVELS, AND CHLOROPHYLL INDEX OF CYLINDROTHECA CLOSTERIUM CULTURES EXPOSED TO AROCLOR 1242

Treatments, Aroclor 1242	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

Source: Keil, J.E., L.E. Priester and S.H. Sandifer. 1971. Polychlorinated biphenyl (Aroclor 1242): Effects of uptake on growth, nucleic acids, and chlorophyll of a marine diatom. Bulletin of Environmental Contamination and Toxicology 6(2):156-159.

Table III.3.3 (from ref. 86)

GROWTH OF *T. pseudonana* AND *D. tertiolecta* AT THREE TEMPERATURES AND THREE CONCENTRATIONS OF PCBS. GROWTH RATES (μ) ARE EXPRESSED AS DIVISIONS PER DAY AND WERE CALCULATED ACCORDING TO THE EXPRESSION $\mu = (3.5)(\ln n_t - \ln n_{t_0})/(t - t_0)$ WHERE $t - t_0$ REPRESENTS ELAPSED TIME IN HOURS AND n_t AND n_{t_0} ARE THE POPULATION DENSITIES AT TIMES t AND t_0, RESPECTIVELY (EPPLEY & STRICKLAND, 1968). THE VALUES SHOWN (MEANS OF THREE REPLICATES) ARE GROWTH RATES FOR THE FIRST 72 H AT 18°C AND 25°C AND FOR THE FIRST 120 H AT 12°C.

Temperature	*[PCB] in ppm*	*Growth rate of* T. pseudonana	*% control*	*Growth rate of* D. tertiolecta	*% control*
12°C	0·0	1·43	100	0·74	100
12°C	0·1	1·57	109	0·75	101
12°C	10·0	0·60	42	0·79	107
18°C	0·0	2·34	100	1·22	100
18°C	0·1	2·15	84	1·23	101
18°C	10·0	1·31	56	1·18	97
25°C	0·0	2·57	100	1·66	100
25°C	0·1	2·59	101	1·66	100
25°C	10·0	2·13	83	1·69	102

GROWTH OF *R. setigera* AT TWO TEMPERATURES AND FOUR CONCENTRATIONS OF PCBS. GROWTH RATES ARE EXPRESSED AS DIVISIONS PER DAY AND WERE CALCULATED AS SHOWN IN TABLE I. THE VALUES SHOWN (MEANS OF THREE REPLICATES) ARE GROWTH RATES FOR THE FIRST 192 H AT 10°C AND FOR THE FIRST 144 H AT 15°C.

Temperature	*[PCB] in ppm*	*Growth rate of* R. setigera	*% control*
10°C	0·0	0·60	100
10°C	0·1	0·49	82
10°C	1·0	0·46	77
10°C	10·0	0·49	82
15°C	0·0	0·86	100
15°C	0·1	0·81	95
15°C	1·0	0·75	87
15°C	10·0	0·73	85

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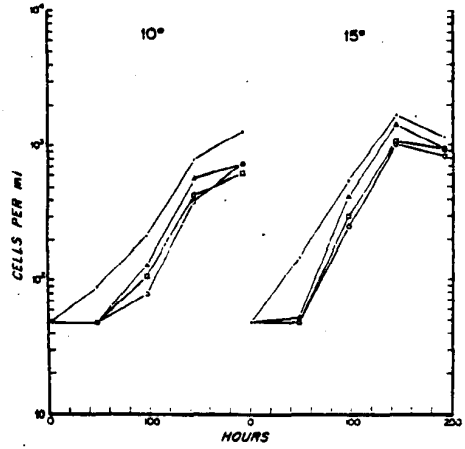

Fig. 2. Growth of *R. setigera* at two different temperatures and with four PCB concentrations. (●——●) = 0 ptm of PCBs; (Δ——Δ) = 0·1 ptm; (□——□) = 1·0 ptm; (○——○) = 10 ptm. Data points are the means of three replicates.

Fig. III.3.1 (from ref. 86)

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that Dunaliella tertiolecta, an estuarine and marine green alga, was not affected at any of the temperatures or concentrations of Aroclor 1254 that inhibited the marine diatoms (86: Table III.3.3). Mosser et al. (87,88) also found differences in sensitivity to Aroclor 1254 between marine diatoms and the green alga D. tertiolecta. This alga was not affected by concentrations of Aroclor 1254 up to 1,000 ppb, while 10 ppb and 25 ppb affected the diatoms Skeletonema costatum and Thalassiosira pseudonana, respectively (see Figure III.3.2). The viability of T. pseudonana showed a decrease at 50 and 100 ppb Aroclor 1254. The sensitivity levels should be considered as maxima, since evaporation loss and adsorption of the PCBs to the walls of the flask have not been taken into account.*

Further investigations of geographic differences in sensitivity to PCBs compared estuarine and oceanic colonies of the same diatom species (89). Three species of diatoms were isolated from the relatively stable Sargasso Sea and from the more variable environments of continental shelf and estuarine areas. The diatoms tested were Thalassiosira pseudonana, Fragilaria pinnata and Bellerochia sp. All clones grew more slowly with 10 ppb Aroclor 1254 added, but clones from the Sargasso Sea were more sensitive than estuarine and continental shelf isolates (Table III.3.4). The clone from the continental shelf was, in turn, more sensitive

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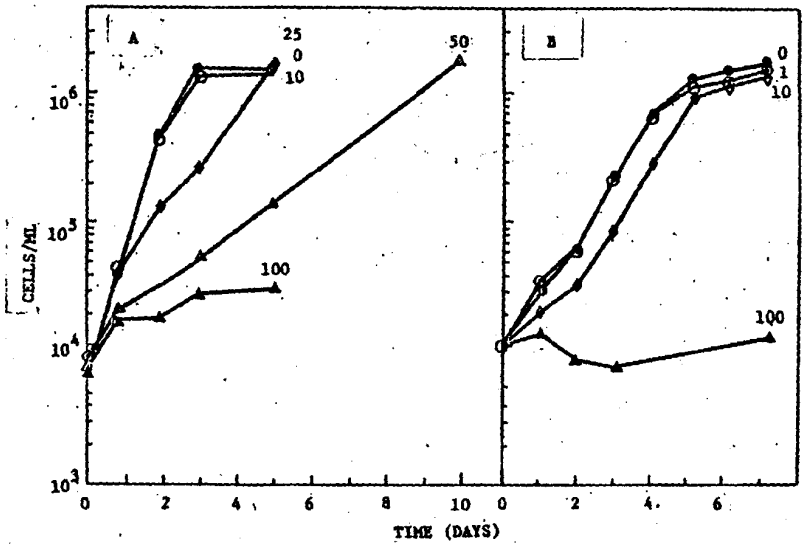
*This comment applies to all the experiments with phytoplankton reviewed in this section, since the actual concentration of PCBs in the culture medium does not appear to have been measured in any of the studies.



Source: Mosser, J. L., N. S. Fisher, T.-C. Teng, and C. F. Wurster, 1972. Polychlorinated biphenyls: toxicity to certain phytoplankters. Science 175(4018):191-192.

MONS 064250

Effects of Aroclor

pseudonas

94

Table III-3.4 (from ref. 90)

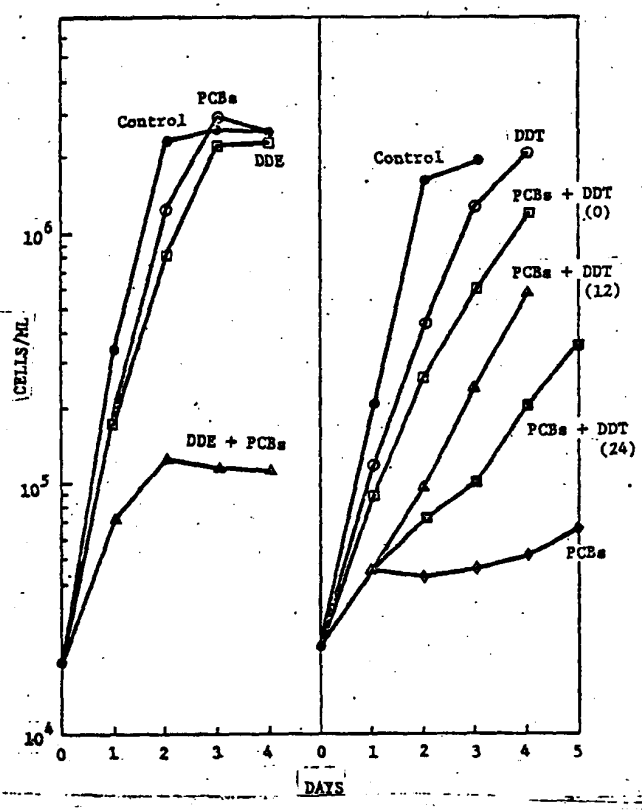
RATIOS OF MEAN CELL DENSITIES FROM 10ppb AROCLOR 1254-TREATED CONTROL CULTURES AT FIVE SAMPLE TIMES

	HOURS AFTER INOCULATION				
	0	47.5	70	95	118
Thalassiosira pseudonana clones					
3H (estuarine)	1.00	0.694	0.365	0.676	0.976
7-15 (continental shelf)	1.00	0.544	0.368	0.520	0.844
13-1 (oceanic)	1.00	0.422	0.241	0.172	0.341
Fragilaria pinnata clones					
0-12 (estuarine)	1.00	0.824	0.909	0.932	0.590
13-3 (oceanic)	1.00	0.688	0.707	0.269	0.387
Bellerochia clones					
Say-7 (estuarine)	1.00	0.207	0.064	0.036	0.035
SD (oceanic)	1.00	0.074	0.020	0.013	0.005

than its counterpart from the estuary.

Synergistic effects occurred when DDE and Aroclor 1254 were used simultaneously with the diatom Thalassiosira pseudonana (90). At concentrations of 10 ppb Aroclor 1254 or 100 ppb DDE, minor growth inhibition occurred. However, used at the same concentrations simultaneously, there was substantial inhibition of growth, with still greater inhibition at higher concentrations (see Figure III.3.3). However, the effects of DDT were opposite to those of DDE. At 50 ppb, Aroclor 1254 almost stopped diatom growth. When 500 ppb DDT was added simultaneously with 50 ppb Aroclor 1254, growth was restored to two-thirds of the control (see Figure III.3.3). Lower DDT concentrations reversed Aroclor 1254 inhibition to a lesser extent. If DDT was added 12 or 24 hours after Aroclor 1242 inhibited diatom cultures, growth was again restored. Hence, co-precipitation of the inhibitor PCB by DDT in the medium was not the mechanism of reversal, but some intracellular reaction must be involved (90).

The uptake of carbon by natural plankton communities was affected by Aroclor 1242, Aroclor 1254, and 2,4'-dichlorobiphenyl (91). The plankton communities were dominated by the diatom genera Ceratulina, Chaetoceros, Biddulphia, Eupodiscus, Leptocylindrica, Melosira, Nitzschia, Rhizosolenia, and Thalassionema. Two dinoflagellate genera were also generally present: Peridinium and Ceratium. Sensitivity to Aroclor 1242 and 1254 was apparent at 1-2 ppb and to the dichlorobiphenyl at 7 ppb (see Figure III.3.4). In natural phytoplankton communities all stages of growth are represented through various phytoplankton species. Hence, while



Interactions among PCBs, DDE and DDT in Thallassiosira pseudonana.

Figure III.3.3 (from ref. 90

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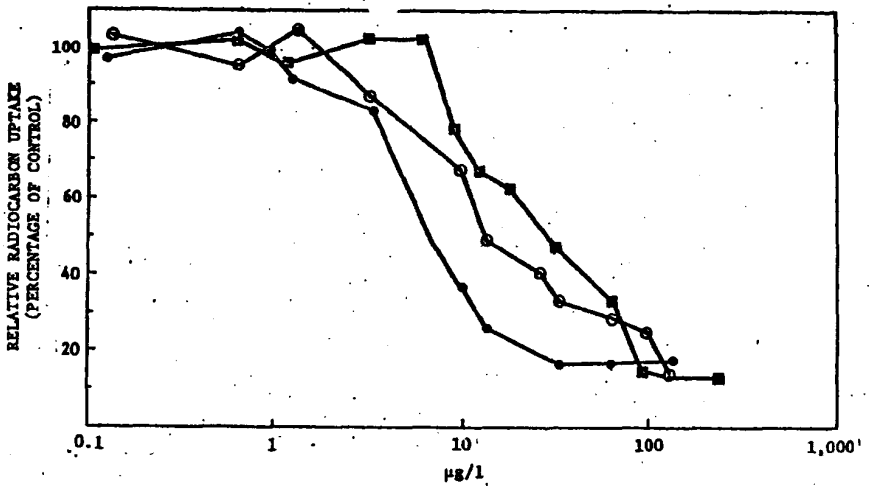


2,4'-dichlorobiphenyl (■); 'Aroclor 1242' (●) and 'Aroclor 1254' (○).

Adapted from: Moore, S. A., Jr. and R. C. Harriss. 1972. Effects of polychlorinated biphenyl on marine phytoplankton communities. Nature 240: 356-358.

Effect of organochlorines on in situ radiocarbon uptake by marine phytoplankton communities.

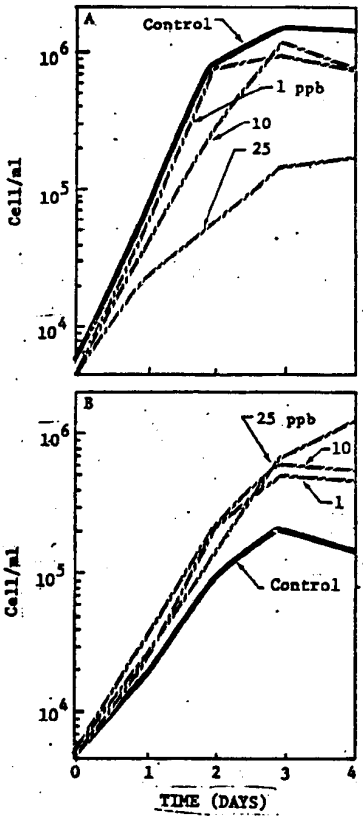
Figure III.3.4 (from ref. 91)

MONS 064254

overall photosynthesis diminished in the community exposed to PCBs, the precise species and/or active site cannot be identified. Nor were changes in species composition investigated.

In a key experiment, Mosser et al. (88) tested the effects of PCBs on growth of a resistant species (the green alga D. tertiolecta) and a sensitive species (the diatom T. pseudonana) in a mixed culture. Although there were only slight effects on growth of either species at concentrations of 1 or even 10 ppb of Aroclor 1254 when cultured alone, there was a large change in the species ratio when the two species were exposed together to 1 ppb (Figures III.3.5 and III.3.6). Thus Aroclor 1254 significantly diminished the competitive success of the diatom and increased that of the alga, even at concentrations well below that required for detectable effects on either alone (88). Similar effects on competition between sensitive and resistant species were demonstrated at still lower PCB levels (0.1 ppb) by Fisher et al. (92). Figure III.3.7 shows the effect of 0.1 ppb Aroclor 1254 on T. pseudonana in continuous culture with the resistant species D. tertiolecta. Table III.3.5 shows the effects of 0.1 ppb Aroclor 1254 on a natural phytoplankton community in continuous culture. Not only was the population of the sensitive T. pseudonana markedly reduced, but the diversity of species in the culture was also decreased (92, Table 3).

Such changes in species composition are potentially the most important effects of PCBs on phytoplankton. Most species of zooplankton graze selectively, often choosing their food on the basis of size or shape. Studies on oysters, barnacles, clams,



Growth of (A) Thallassiosira pseudonana and (B) Dunaliella tertiolecta in mixed cultures with Aroclor 1254 added.

Figure III.3.5 (from ref. 88)

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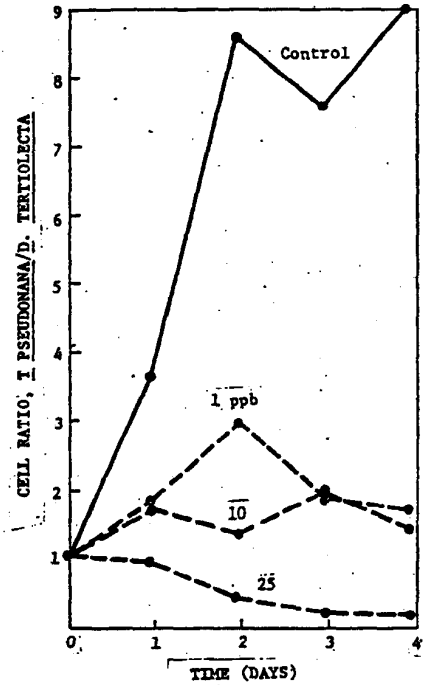


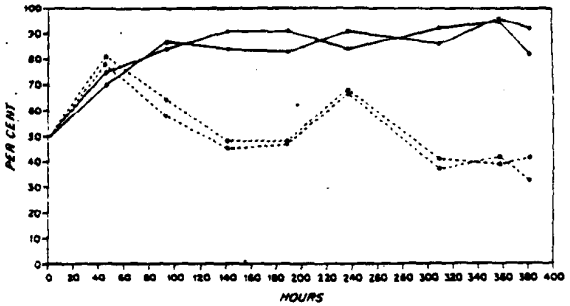
Species ratios in mixed cultures treated with Aroclor 1254.

Figure III.3.6 (from ref. 88)

MONS 064257



Growth in continuous pure culture of *T. pseudonana* (•———•) and *D. tertiolecta* (o———o) with (- - -) or without (———) 0.1 ppb of PCB. Data points are means from duplicate cultures. A single classification analysis of variance [37] showed that treated and control culture values did not differ significantly for either species.



Continuous cultures of gnotobiotic communities containing *T. pseudonana* and *D. tertiolecta*, showing percent of total cell counts represented by *T. pseudonana* growing with (- - -) or without (———) 0.1 ppb of PCB. Duplicate values are shown.

Figure III.3.7 (from ref. 92)

Table III.3.5 (from ref. 92)

Growth of the Three Dominant Species in Natural Phytoplankton Communities in Continuous Culture[a]

		T. pseudonana (X 10^3 cells/ml)		*Chaetoceros* sp. (X 10^3 cells/ml)		*S. costatum* (X 10^3 cells/ml)	
	Hours	0 ppb	0.1 ppb	0 ppb	0.1 ppb	0 ppb	0.1 ppb
A	0	4.0 ± 1	4.0 ± 0	5	4	3	1
	146	23.5 ± 0.5	2.5 ± 1.5	195	335	23	12
B	41	2.0 ± 0.5	2.3 ± 1.3	32	12	4	3
	184	11.5 ± 5.5	3.0 ± 2	469	378	7	7

[a]Initial and final cell densities are means from duplicate treated and control cultures. The dilution rate for experiment A was 33 ± 3% of the volume/24 hr; for experiment B was 47 ± 4% of the volume/24 hr.

and copepods have shown that the dietary requirements of herbivores are not satisfied by all species of algae (88). The replacement of sensitive diatoms by resistant green algae in marine phytoplankton communities could thus have profound effects on their consumers and hence on the distribution and abundance of many animal populations higher in the food web (83, 88). In experiments with single species, effects on growth have been demonstrated at PCB concentrations as low as 0.1 ppb (84, 86). The experiments reported in refs. 88 and 92 show that effects on species composition and hence on foodwebs are to be expected at still lower levels (83, 92).

Although most experiments with phytoplankton have been carried out with Aroclor 1254, Aroclor 1242 inhibited growth of Scenedesmus at a concentration of only 0.1 ppb (84), and in the experiment with a mixed culture conducted by Moore and Harriss (91), Aroclor 1242 was more active than Aroclor 1254 (Figure III.3.4). Hence tetra- and trichlorobiphenyls are probably at least as toxic to phytoplankton as higher chlorobiphenyls.

III.4. Effects on Aquatic Invertebrates

Effects of PCBs on aquatic invertebrates have recently been reviewed by Walker, Nebeker, and Hansen (73-75).

III.4.1. Effects on fresh-water organisms

Fresh-water crustaceans are very sensitive to low concentrations of PCBs. Table III.4.1 summarizes the results of static and continuous flow bioassays conducted with Daphnia magna using eight Aroclor mixtures (93). The mixtures appeared much more toxic in continuous-flow conditions due to continual replacement

CALCULATED 2- AND 3-WEEK LC50 AND 16% REPRODUCTIVE IMPAIRMENT VALUES FOR DAPHNIA MAGNA SUBJECTED TO AROCLOR 1248 AND AROCLOR 1254 IN CONTINUOUS-FLOW CONDITIONS AND EIGHT AROCLORS IN STATIC CONDITIONS AT 18 ±1 C

Aroclor (PCB)	3-wk LC50 (µg/liter)	Confidence limits (0.05)	Reproductive impairment (µg/liter) 50%	16%
		Static test conditions		
A-1221	180	158.0-205.0	125	89
A-1232	72	62.6- 82.8	66	53
A-1242	67	55.4- 81.0	63	48
A-1248	25	21.4- 29.2	24	16
A-1254	31	25.8- 37.2	28	18
A-1260	36	27.7- 46.8	33	22
A-1262	43	37.0- 49.9	41	24
A-1268	253	222.0-288.0	206	162
		Continuous-flow test conditions		
A-1248	2.6 (2-wk LC50)		2.1	1.0
A-1254	1.8 (2-wk LC50)		1.1	0.48
A-1254	1.3 (3-wk LC50)		1.3	1.0

Source: Nebeker, A.V. and F.A. Puglisi. 1974. Effect of Polychlorinated Biphenyls (PCBs) on Survival and Reproduction of Daphnia, Gammarus, and Tanytarsus. Transactions of the American Fisheries Society 103(4):722-728.

Table III.4.1 (from ref. 93)

of fresh PCBs. In static conditions much of the PCBs added to the test water was lost to the air, with some lost to bacteria, algae, waste materials, container surfaces, and animal tissues. The differences shown in Table III.4.1 between results in static and continuous-flow bioassays show how critical the test methodology can be for hydrophobic chemicals such as PCBs (74).

In the static tests with Daphnia magna, Aroclor 1248 was the most toxic of the eight mixtures tested; the 3-week LC_{50} was 25 µg/l. In the continuous-flow tests, Aroclor 1254 was more toxic, with a 3-week LC_{50} of 1.3 µg/l. No reproduction occurred at 3.8 µg/l, and reproduction was significantly impaired at concentrations below 1.0 µg/l (Table III.4.1).

The toxicity of Aroclor 1254 to Daphnia magna is significantly enhanced by the presence of DDT, although the effects of the two compounds are less than additive (94). In a static bioassay system, the two-week LC_{50}s for DDT and Aroclor 1254 to Daphnia were 0.67 ppb and 24.0 ppb, respectively. Addition of 12 ppb Aroclor increased the toxicity of DDT by approximately one-third, and addition of 0.5 ppb DDT doubled the toxicity of Aroclor 1254 (Table III.4.2).

The fresh-water scud Gammarus pseudolimnaeus was also studied in continuous-flow test systems to determine the effects of Aroclors 1242 and 1248 on survival and reproduction (93). Concentrations as low as 8.7 µg/l Aroclor 1242 and 5.1 µg/l Aroclor 1248 caused approximately 50 percent mortality in 60 days' exposure and seriously affected reproduction (Table III.4.3).

Table III.4.4 summarizes the results of static and continuous-flow tests with five other species. The glass shrimp Palaemonetes

Table III.4.2 (from ref. 94)

The concentrations (ppb) of p,p' DDT and Aroclor 1254 alone and in combination that affect survival (LC_{50}) and reproduction (EC_{50}) of Daphnia magna in a 14 day test.

Toxicant Combination	Acute Toxicity 14-day LC_{50}		Reproductive Inhibition EC_{50} (ppb)					
			Total Young		Average Brood Size		Percentage of Days Reproducing	
	mean	C.L.*	mean	C.L.	mean	C.L.	mean	C.L.
p,p' DDT only	0.67	(0.65- 0.69)	0.50	(0.48- 0.52)	0.61	(0.58- 0.64)	0.75	(0.71- 0.79)
DDT with 12 ppb Aroclor 1254	0.40	(0.38- 0.41)	0.30	(0.28- 0.32)	0.45	(0.43- 0.48)	0.55	(0.52- 0.58)
Aroclor 1254 with 0.75 ppb DDT	4.0	(3.80- 4.20)	1.8	(1.69- 1.91)	6.0	(5.82- 6.18)	9.0	(8.65- 9.36)
Aroclor 1254 with 0.50 ppb DDT	12.0	(11.54-12.48)	10.0	(9.17-10.90)	13.0	(11.92-14.17)	11.0	(10.09-11.99)
Aroclor 1254 with 0.30 ppb DDT	14.0	(13.46-14.56)	13.0	(12.26-13.78)	19.0	(18.27-19.76)	19.0	(17.92-20.14)
Aroclor 1254 only	24.0	(23.08-24.96)	19.0	(17.93-20.14)	23.0	(22.12-23.92)	25.0	(23.81-26.25)

*Confidence limits for p = 0.05.

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Table III.4.3 (from ref. 93)

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
0.5	71	20
0.2	73	11
0.0	64	11

Table III.4.5 (from ref. 93)

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

Table III.4.4 (from ref. 95)

Aroclor and DDT toxicity to invertebrates.

Compound	Organism[1]	Bioassay type[2]	Exposure (days)	LC_{50} (μg/l)
Aroclor 1242	Crayfish	static	7	30
Aroclor 1254	Crayfish	static	7	100
Aroclor 1254	Crayfish	continuous-flow	7	80
DDT	Crayfish	static	4	100
Aroclor 1242	Scud	continuous-flow	4	10
Aroclor 1242	Scud	continuous-flow	10	5.0
Aroclor 1248	Scud	static	4	52
Aroclor 1254	Scud	static	4	2,400
DDT	Scud	static	4	3.2
DDT	Scud	continuous-flow	5	0.6
Aroclor 1254	Glass shrimp	continuous-flow	7	3.0
DDT	Glass shrimp	static	5	1.0
DDT	Glass shrimp	continuous-flow	5	1.3
Aroclor 1242	Dragonfly	static	7	800
Aroclor 1254	Dragonfly	static	7	1,000
Aroclor 1242	Damselfly	continuous-flow	4	400
Aroclor 1254	Damselfly	continuous-flow	4	200
DDT	Damselfly	static	4	56

[1] Crayfish (*Orconectes nais*), Scud (*Gammarus fasciatus*), Glass shrimp (*Palaemonetes kadiakensis*), Dragonfly (*Macromia sp.*), Damselfly (*Ischnura verticalis*).

[2] Temperature, 15.6°C; alkalinity, 35 ppm; pH, 7.1.

MONS 064265

kadiakensis was very sensitive to Aroclor 1254, with a 7-day LC_{50} of 3 µg/l (95).

In a comparative toxicity test with five individual chlorobiphenyls under static conditions, Mayer et al. (96) found that 2,3,4'-trichloro-biphenyl was slightly more toxic to G. pseudolimnaeus than representative di-, tetra-, penta-, or hexachlorobiphenyls; the 96-hour LC_{50} valves were 70, 100-120, 110, 210 and 150 µg/l respectively. Under static test conditions, the toxicities of Arcolors 1016 and 1242 to D. magna were similar: no young survived after 3 weeks' exposure to 125 µg/l of either mixture (74, 97).

Aquatic insects also appear to be very sensitive to prolonged exposure to PCBs (74). The midge Tanytarsus dissimilis was tested through its full life cycle (93) 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 III.4.5). 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 ug/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 (93).

Studies by Sanders and Chandler (98) 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 for up to 21 days without significant mortality. However, in similar experiments, 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.

III.4.2 Effects on estuarine organisms

PCBs are acutely toxic to estuarine organisms (74,99-101). For Aroclors 1016, 1242 and 1254, the 48-hour or 96-hour LC_{50} values for brown shrimps Penaeus aztecus, pink shrimps P. duorarum, and grass shrimps Palaemonetes pugio fall into the range 9-32 µg/l (74,99,100). Exposure to 10 µg/l Aroclor 1016 for 96 hours caused 43% mortality in brown shrimps and 38% mortality in grass shrimps (100), whereas the same concentration of Aroclor 1254 caused no mortality in pink shrimps in the same period (102). The acute toxicities of Aroclors 1016 and 1254 to oysters Crossostrea virginica appeared to be similar (99,100).

Laboratory bioassays lasting longer than two weeks have demonstrated that acute bioassays underestimate the toxicities of Aroclors 1016 and 1254. Aroclor 1254 is toxic to commercially valuable penaeid shrimps and grass shrimps at concentrations as low as 0.9-1.4 µg/l (102-105; Tables III.4.6, III.4.7). Exposed shrimps are particularly sensitive to salinity stress (103) and apparently to viral disease (106,107). Aroclor 1254 also decreases

Table III.4.6 (from ref. 102)

RESULTS OF CHRONIC BIOASSAYS WITH AROCLOR 1254 AND THE PINK SHRIMP PENAEUS DUORARUM IN FLOWING WATER

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

-- No data
[a] Average of at least three determinations.
[b] Student's t-test.
[c] Chi-square.

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Table III.4.7 (from ref. 104)

MORTALITY AND ACCUMULATION OF AROCLOR 1254 IN *Palaemonetes pugio**

Test Conc. (µg/l)	Days Exposed	Average Mortality (%)**	Body Conc. (mg/kg)	Concentration Factor
CONTROL	7	4(0 - 20)	0.1	-----
0.17	7	8(0 - 40)	1.3	7600
0.62	7	4(0 - 20)	5.4	8700
9.1	7	60(20 - 80)***	65.0	7100
CONTROL	16	25(0 - 50)	<0.1	-----
1.3	16	40(0 - 100)	18.0	14000
4.0	16	45(25 - 50)***	27.0	6700
12.5	16	55(50 - 75)***	46.0	3700

*All exposures were conducted in flowing seawater: salinity and temperature ranges were 22 to 28‰ and 17 to 28°C.

**5 replicates per concentration: at least 4 shrimp per replication.

***Significant at P >0.05.

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the growth rates of oysters at concentrations as low as 4 ug/l (111; Figure III.4.1).

III.4.2 Effects on a marine invertebrate community

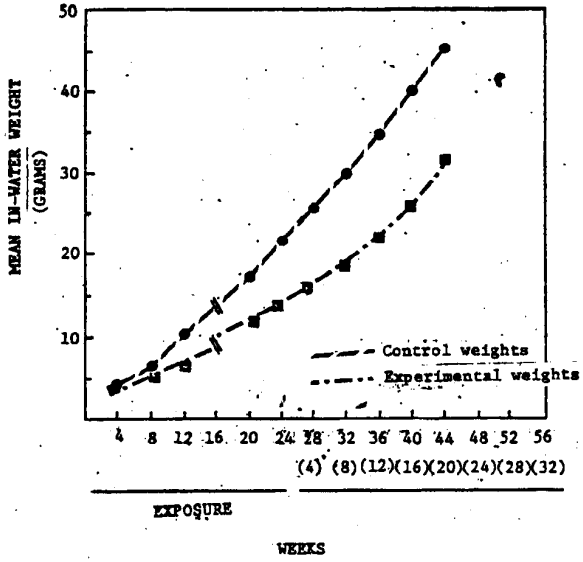
Hansen (109) showed that Aroclor 1254 affected the composition of communities of estuarine animals which developed from planktonic larvae in seawater flowing through aquaria in the laboratory. In control aquaria, the communities were dominated by arthropods, primarily the amphiped Corophium volutator. In aquaria receiving Aroclor 1254 at 1 or 10 µg/l, the number of arthropods decreased and the communities were dominated by tunicates (a group of animals characteristic of polluted waters). The numbers of amphipods, bryozoans, crabs, and molluscs were also reduced in these treated aquaria. These effects were manifested even in aquaria receiving only 0.1 µg/l of Aroclor 1254: the number and variety of molluscs were markedly reduced and the number of tunicates was increased (Table III.4.6). This experiment is important because it shows that PCBs can have marked effects on species composition and biological diversity at levels much lower than those required to demonstrate effects with single species.

III.5. Effects on Fish

Effects of PCBs on fish have recently been reviewed by Walker, Nebeker, and Hansen (73-75).

III.5.1. Lethal concentrations of PCBs to fish

Table III.5.1 summarizes measurements of the acute and chronic toxicities of eight Aroclor mixtures to four species of fish (73, citing data from refs. 95, 96). As in the case of the aquatic invertebrates, PCBs do not appear very toxic in short-term static



Change in oyster weight after exposure to 5 ppb Aroclor 1254 for 24 weeks.

Figure III.4.1 (from ref. 108)

Table III.4.6 (from ref. 109)

Species and total number of animals collected from the effluents of 10 control aquaria and 10 aquaria contaminated for four months with 0.1, 1 or 10 µg/l Aroclor 1254.

Taxa	Control	0.1 µg/l	1 µg/l	10 µg/l
Annelida				
Eupomatus dianthus	1	1	0	0
E. protulicola	0	1	1	0
Neanthes succinea	1	2	2	0
Total	3	4	3	0
Arthropoda				
Balanus sp.	19	2	2	1
Caprella sp.	0	8	4	0
Clibanarii tricolor	0	1	0	0
Corophium volutator	7	15	32	1
Eurypanopeus depressus	5	2	5	0
Neopanope texana	10	6	3	0
Pagurus longicarpus	1	0	0	0
Pinnixa chaetopterana	1	0	0	0
Upogebia affinis	1	1	0	0
Decapod zoea, unident. sp.	0	0	1	0
Portunidae, unident. sp.	2	1	0	0
Pycnogonidae, unident. sp.	0	0	3	0
Total	37	36	50	2
Chordata				
Botrichobranchus pilularis	3	0	0	0
Branchiostoma caribaeum	1	0	1	0
Molgula manhattensis	72	103	304	35
Total	76	103	305	35
Coelenterata				
Leptomedusae*, unident. sp.	1	1	1	1
Echinodermata				
Hemipholis elongata	1	0	3	0
Ectoprocta				
*Membranipora tenuis**	1	1	1	0
Mollusca				
Anadara ovalis	1	0	0	0
A. transversa	12	0	4	1
Bittium alternata	0	1	0	0
B. varium	3	0	1	0
Crassostrea virginica	2	0	1	1
Doridella obscura	12	1	2	1
Laevicardium mortoni	1	0	0	0
Mitrella lunata	2	0	2	2
Musculus lateralis	1	0	1	0
Nassarius albus	2	0	0	0
Tagelus divisus	1	2	0	0
Eulimacea, unident. sp.	1	1	1	3
Total	38	5	12	8
Totals: Animals	156	150	375	47
Species	27	18	20	10

* Colonies: Counted as one animal.

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Table III.5.1 Toxicity of PCBs to fish (refs. 73, 95, 96)

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

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Table III.5.1 (continued)

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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bioassays, but are lethal at much lower concentrations on prolonged exposures. The rainbow trout was the most sensitive of the four species listed in Table III.5.1, being killed by levels as low as 3.4 µg/l Aroclor 1248 in 25 days, or 5 µg/l Aroclor 1260 in 10 days. Aroclor 1248 was generally the most toxic mixture tested in prolonged exposures.

In a separate series of tests under static conditions, the 96-hour LC_{50} for Aroclor 1016 was determined to be 650 µg/l for bluegills and 750 µg/l for channel catfish at 18°C; that for Aroclor 1221 was determined to be 230 ppm for bluegills and 3340 µg/l for channel catfish (110). These figures suggest that the acute toxicities of Aroclors 1016 and 1221 are comparable with or greater than those of Aroclor 1248 (Table III.5.1).

In chronic studies with estuarine fish, Aroclor 1016 appeared somewhat less toxic than Aroclor 1254. Spot and pinfish died when exposed for 14-45 days to 5 µg/l of Aroclor 1254 (111), whereas pinfish were only slightly affected by exposure for 42 days to 7 µg/l of Aroclor 1016 and their mortality was not significantly increased until the measured concentrations of Aroclor 1016 reached 13 µg/l (100). The longnose killifish is the most sensitive species tested, being killed by prolonged exposure to 1 µg/l of Aroclor 1254 (105).

Other recent work conducted by Mayer and Johnson has suggested that the toxicity of Aroclor 1016 to fresh-water fish is generally similar to that of Aroclor 1242 (75). Bluegills had a 96-hour LC_{50} of 48-260 µg/l Aroclor 1016; rainbow trout, 135 µg/l; Atlantic salmon, 134 µg/l; and yellow perch, 185 µg/l. On longer exposures, rainbow trout had a 10-day LC_{50} of 39 µg/l for Aroclor

1242 and a 17-day LC_{50} of 49 µg/l for Aroclor 1016. Bluegills had a 15-day LC_{50} of 54 µg/l for Aroclor 1242 and a 35-day LC_{50} for Aroclor 1016 (75).

All of the LC_{50} values decreased with higher temperatures and longer exposure to PCBs (Table III.5.1). An increase in temperature from 20° to 27°C approximately doubled the toxicity of Aroclor 1248.

Recent studies have shown that young fish are more sensitive than older fish (75, 112). Ninety-six-hour LC_{50} values for newly hatched fathead minnows were 15 µg/l for Aroclor 1242 and 7.7 µg/l for Aroclor 1254. After 60 days, half were dead at 8 8 µg/l Aroclor 1242 and 4.6 µg/l Aroclor 1254. Newly-hatched young were the most sensitive life stage. Growth of young fathead minnows was also affected at 2.2 µg/l Aroclor 1248 and none survived above 5.1 µg/l after 30 days. Young flagfish did not survive at concentrations of Aroclor 1248 above 5.1 µg/l and did not grow well above 2.2 µg/l (112).

III.5.2. Effects on fish reproduction

Two 9-month continuous-flow bioassay tests were conducted with fathead minnows to determine the effects of Aroclor 1242 and Aroclor 1254 on survival and reproduction (112). Concentrations of Aroclor 1242 above 10 µg/l were lethal to newly-hatched fry. Reproduction occurred and good egg hatching took place at and below concentrations of 5.4 µg/l, but the number of eggs produced was reduced at all concentrations tested down to 0.9 µg/l (Table III.5.2). Aroclor 1254 appeared more toxic than Aroclor 1242, as fathead minnows did not survive and reproduce at concentrations above 1.8 µg/l (Table III.5.2). Spawning was signifi-

Table III.5.2 (from ref. 112)

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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cantly reduced at 1.8 µg/l; reproduction was good at 0.5 µg/l and lower concentrations. In chronic tests with Aroclors 1248 and 1260, fathead minnows were able to reproduce at concentrations which were acutely toxic to the larvae. Reproduction occurred at and below concentrations of 3 µg/l Aroclor 1248 and 2.1 µg/l Aroclor 1260, but mortality of the newly hatched larvae led to a 20 percent reduction in the population in the second generation at concentrations as low as 0.4 µg/l (113).

Aroclor 1254 also affects reproduction of the sheepshead minnow, an estuarine fish (114, 115). 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 more than 5 ppm of PCBs and increased as the PCB content of the eggs increased (75, 114, 115). However, sheepshead minnows exposed to Aroclor 1016 at levels from 0·3 to 3·0 µg/l of Aroclor 1016 reproduced successfully; although the eggs contained 3-77 ppm of PCBs, fry hatched from them appeared to be unaffected (116). This is one of the few examples in which Aroclor 1016 appears to have been significantly less toxic than other mixtures.

In tests with rainbow trout, fry from eggs containing 2·7 ppm Aroclor 1242 together with 0.09 ppm DDT suffered a 75 percent mortality in the 30 days after hatching. In five other groups of eggs with still lower residues, 10-28 percent mortality was noted within the first 30 days. About 60-70 percent of the fry that survived were deformed (117, 73). Swedish investigators have

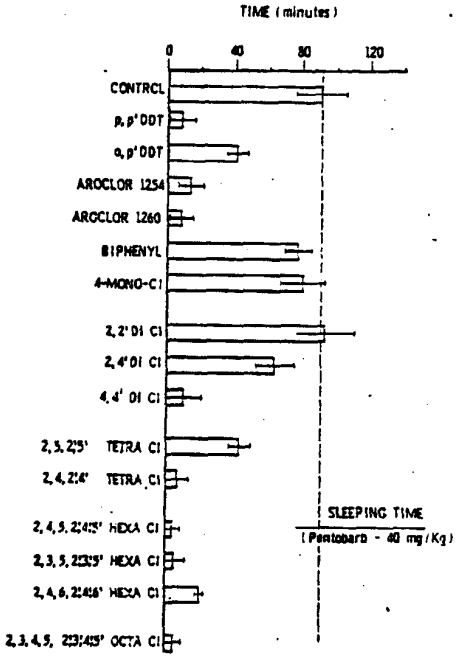
reported a statistically significant association between hatching failure in eggs of Atlantic salmon and PCB residues. Concentrations in the range 0·4 to 1·9 ppm, wet weight (7·7-34 ppm, lipid weight) were associated with mortalities between 16 and 100 percent (118).

Coho salmon eggs hatched two to five days premature when exposed to Aroclor 1254 (119; see Table III.5.3). Yolk-sac utilization was considerably reduced in the exposed eggs. Early hatching was preceded by changes in the egg surface, possibly decreasing the permeability of the chorion to oxygen and thus inducing early hatching (119). Survival following hatching was inversely related to the exposure concentration and time. Early hatching and reduced survival were noted even at the lowest concentration tested, 5 µg/l (Table III.5.3).

In contrast to these reported effects of low levels of PCBs on reproduction in rainbow trout and salmon, no adverse effects were observed on survival, growth, or reproduction of brook trout exposed for 71 weeks to 0·94 µg/l and lower concentrations of Aroclor 1254. Survival and growth of alevins from exposed parents were also unaffected (75, 120).

III.5.3. Other sublethal effects on fish

Inclusion of Aroclor 1254 in fish food at a level of 0·45 ppm resulted in a 52 percent increase in thyroid activity in coho salmon (Figure III.5.1). The lowest detectable stimulation of thyroid activity occurred in salmon ingesting a dose no more than one-thousandth of that required to cause mortality (73, 96). Similar results were obtained in channel catfish exposed to 2·4 ppm



The effect of pretreatment of young male rats with DDT (o,p' and p,p'-isomers), Aroclor 1254 and 1260, biphenyl, and a series of isomerically pure chlorobiphenyls on the sleeping time produced by an injection of 40 mg/kg sodium pentobarbital.

Figure III.10.2 (from ref. 241)

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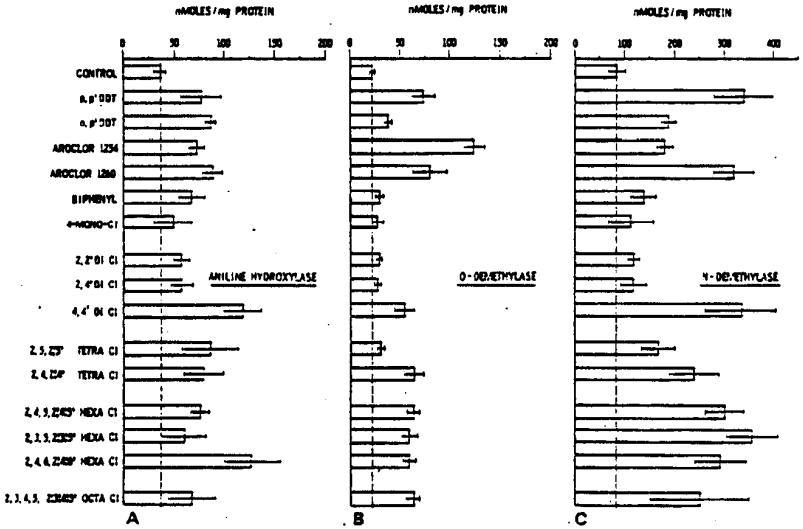
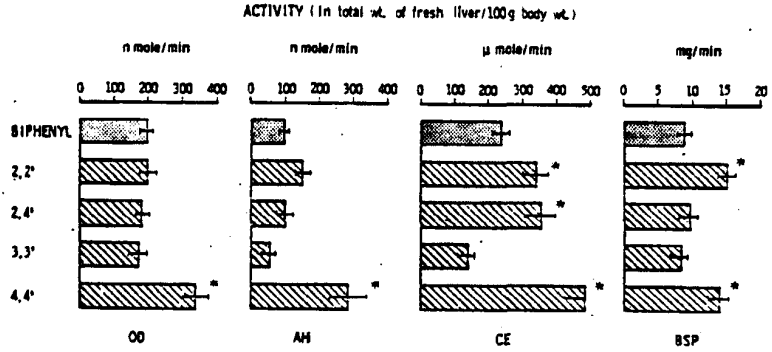


The effect of pretreatment of young male rats with DDT (o,p'- and p,p'-isomers), Aroclor 1254 and 1260, biphenyl, and a series of isomerically pure chlorobiphenyls on hepatic microsomal aniline hydroxylase (A), p-nitroanisole O-demethylase (B), and aminopyrine N-demethylase (C) activities. Activities are expressed as nmoles of product formed/mg microsomal protein/30min incubation.

Figure III.10.3 (from ref. 241)



The effect of pretreatment of young male rates with biphenyl and a series of isomerically pure dichlorobiphenyls on hepatic p-nitroanisole O-demethylase (OD), aniline hydroxylase (AH), carboxylesterase (CE), and sulfobromophthalein-glutathione conjugating enzyme (BSP) activities. Animals were treated by i.p. injection for 3 consecutive days and were killed and assayed 96 hr after the last injection. The values (bars) represent the mean enzymatic activities ± S.D. (lines) of 18 control, biphenyl-treated rats and 6 animals per treated group. The asterisk (*) indicates values statistically different ($p < 0.35$) from biphenyl-treated controls.

Figure III.10.4 (from ref. 241)

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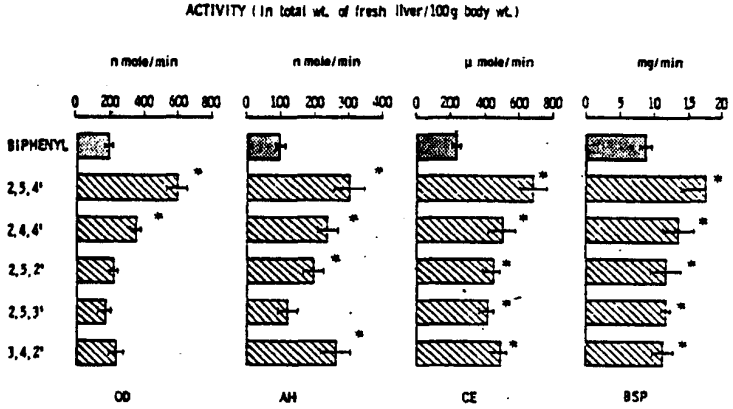
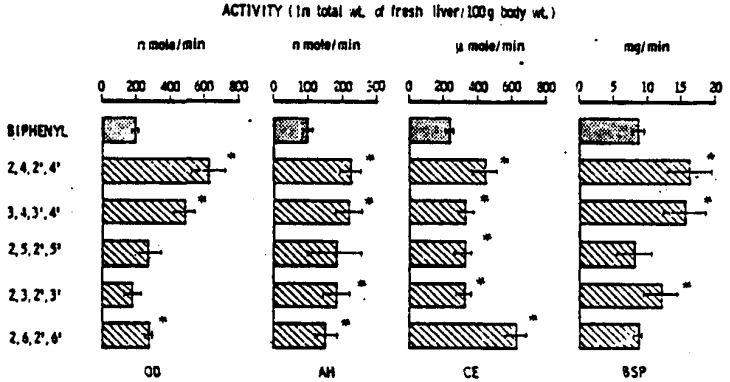


The effects of pretreatment of young male rats with biphenyl and a series of isomerically pure trichlorobiphenyls on hepatic p-nitroanisole O-demethylase (OD), aniline hydroxylase (AH), carboxylesterase (CE), and sulfobromophthalein-glutathione conjugating enzyme (BSP) activities.

Figure III.10.5 (from ref. 241)



The effects of pretreatment of young male rats with biphenyl and a series of isomerically pure tetrachlorobiphenyls on hepatic p-nitroanisole O-demethylase (OD), aniline hydroxylase (AH), carboxylesterase (CE), and sulfobromophthalein-glutathione conjugating enzyme (BSP) activities.

Figure III.10.6 (from ref. 241)

chlorine atoms were not as important. These results, with the exception of the marked effects obtained following treatment with 4,4'-dichlorobiphenyl, confirmed the observations of other investigators who have found that penta-, hexa- and octa-chlorobiphenyls had greater enzyme-inducing potential than did low chlorine-containing biphenyls (205, 224, 239).

The influence of a series of di-, tri-, and tetrachlorobiphenyls on the selected enzymatic functions are shown in Figures III.10.4-6, respectively. With each series of isomers, a chlorine atom on the 4-position caused a more marked induction of hepatic drug-metabolizing enzyme activities than did a chlorine atom at any other position. As one increased the degree of chlorination, subsequent substitution at the 2-position was next in importance followed by substitution at the 3-position. The results conclusively demonstrated that not all isomers of the mono-, di-, tri-, and tetrachlorobiphenyls possess the same toxicologic properties, the position of chlorination being as important as degree of chlorination (242).

Fujita et al. (239) also noted high specific activities of several isomers with 4,4'-substitution, including especially 3,4,3',4'-tetraCB, 2,4,5,3',4'-pentaCB, and 3,4,5,3',4',5'-hexaCB.

As a general summary of these results, for the mono-oxygenases closely associated with the hepatic endoplasmic reticulum, enhanced induction of activity was observed with highly chlorinated biphenyls and by low chlorine-containing congeners having chlorine atoms substituted at the 4- and 4'-positions irrespective of chlorination at

other positions. For those enzymes less discretely localized in the hepatocyte, the position of the chlorine atoms appeared to be less important (242).

III.10.5. Enzyme Induction by Aroclor 1016

Three studies have compared the activity of Aroclor 1016 in inducing liver enzymes with that of more highly chlorinated mixtures. Iverson et al. (243) compared the responses of rats after 21 daily oral doses of 1, 10 and 100 mg/kg of Aroclors 1016 and 1242. In male rats the responses were generally similar and if anything slightly larger for Aroclor 1016 (Table III.10.5): Aroclor 1016 caused significant increases in body weight gain, liver-to-body-weight ratio, and activity of aniline hydroxylase and aminopyrine demethylase at doses as low as 1 mg/kg, and increases in cytochrome P450 at 10 mg/kg. In female rats, by contrast, neither Aroclor caused significant changes at 1 mg/kg and the effects of Aroclor 1242 were more pronounced on all parameters measured except P450 at 10 and 100 mg/kg (Table III.10.6). Neither mixture had significant effects at 0.1 mg/kg (243).

Bickers et al. (244) compared the effects of Aroclors 1016 and 1254 on liver enzymes of male rats following six daily doses of 25 mg/kg. The effects of Aroclor 1254 were larger than those of Aroclor 1016 on each of the six enzyme functions measured (Table III.10.7) and Aroclor 1016 did not have significant effects on aniline hydroxylase or cytochrome b_5. The contrast between this result and the strong induction of aniline hydroxylase by Aroclor 1016 in

Table III.10.5 (from ref. 243)

Effect of Aroclor 1016 on male rats after daily oral dosing for 21 days

	DOSE 1016				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	74.0±4.6	90.7±2.8 **	83.1±4.1	90.4±4.4 **	122
LIVER % BODY WEIGHT	4.11±0.08	4.48±0.04 **	4.66±0.07 **	6.04±0.10 **	147
P_{450} nm/mg protein	0.92±0.04	0.90±0.05	1.43±0.11 **	1.76±0.17 **	191
ANILINE HYDROXYLASE µm/hr/g	2.09±0.10	2.45±0.13 **	4.50±0.35 **	5.60±0.28 **	269
AMINOPYRINE N-DEMETHYLASE µm/hr/g	1.04±0.11	1.44±0.10 **	2.39±0.19 **	2.56±0.20 **	246
LIVER PORPHYRINS µm/g liver	0.18±0.01	0.20±0.03	0.16±0.02	0.44±0.04 **	244

** P<0.05

Effect of Aroclor 1242 on male rats after daily oral dosing for 21 days

	DOSE 1242				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	74.7±5.2	67.0±8.7	75.0±3.4 #	74.0±6.6	100
LIVER % BODY WEIGHT	3.97±0.06	4.26±0.06 **	4.64±0.11 **	6.83±0.18 **	172
P_{450} nm/mg protein	0.92±0.04	1.03±0.05 #	1.29±0.05 **	1.77±0.09 **	192
ANILINE HYDROXYLASE µm/hr/g	1.81±0.07	2.11±0.11 **	3.81±0.17 **	5.75±0.39 **	318
AMINOPYRINE N-DEMETHYLASE µm/hr/g	1.71±0.21	2.11±0.15	3.37±0.28 **	3.85±0.21 **	225
LIVER PORPHYRINS µm/g liver	0.21±0.03	0.19±0.02	0.30±0.05	1.07±0.11 **	510

** P<0.05.

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Table III.10.6 (from ref. 243)

Effect of Aroclor 1016 on female rats after daily oral dosing for 21 days

	DOSE 1016				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control
BODY WEIGHT GAIN (g)	29.2±2.7	30.5±1.8	35.5±2.4	33.6±1.9	115
LIVER % BODY WEIGHT	3.98±0.23	4.05±0.08	4.45±0.08	5.73±0.14 **	144
P_{450} nm/mg protein	0.62±0.08	0.77±0.05	0.78±0.04	1.10±0.03 **	177
ANILINE HYDROXYLASE µm/hr/g	1.10±0.08	1.13±0.09	1.23±0.13	2.48±0.19 **	226
AMINOPYRINE N-DEMETHYLASE µm/hr/g	0.40±0.02	0.44±0.02	0.38±0.04	0.70±0.05 **	175
LIVER PORPHYRINS µm/g liver	0.21±0.02	0.14±0.01	0.24±0.05	1.14±0.20 **	543

** P<0.05

Effect of Aroclor 1242 on female rats after daily oral dosing for 21 days

	DOSE 1242				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	28.7±2.3	28.0±3.7	35.3±3.7	18.3±5.5 **	64
LIVER % BODY WEIGHT	3.63±0.13	3.71±0.11	4.19±0.09 **	6.18±0.22 **	170
P_{450} nm/mg protein	0.85±0.05	0.82±0.03	1.14±0.04 **	1.50±0.05 **	177
ANILINE HYDROXYLASE µm/hr/g	1.01±0.02	1.08±0.03	1.84±0.10 **	3.01±0.23 **	298
AMINOPYRINE N-DEMETHYLASE µm/hr/g	0.45±0.01	0.48±0.05	0.81±0.02 **	1.12±0.12 **	249
LIVER PORPHYRINS µm/g liver	0.20±0.04	0.23±0.02	0.44±0.06 **	1.76±0.30 **	871

** P<0.05

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Table III.10.7 (from ref. 244)

Changes in microsomal hemoprotein levels and N-demethylase and hydroxylase activities in response to Aroclor 1254 and Aroclor 1016[1]

Measurement	Controls	Aroclor 1016	Aroclor 1254
Mg microsomal protein/ g liver, wet weight	25.14 ±0.85	27.07 ±0.58	28.88[2] ±0.88
Aniline hydroxylase, nmoles p-aminophenol/mg protein/hr	36.42 ±1.55	35.83 ±1.90	48.06[2] ±2.82
Ethylmorphine N-demethylase, umoles HCHO/mg protein/hr	0.349 ±0.022	0.498[2] ±0.036	1.075[2] ±0.056
Cytochrome P-450, nmoles /mg protein	0.637 ±0.027	0.884[2] ±0.051	1.776[2] ±0.109
Cytochrome b_5, nmoles /mg protein	0.399 ±0.019	0.423 ±0.009	0.561[2] ±0.014
Zoxazolamine paralysis time, minutes	549 ±58	275[2] ±13	0[2,3]
Hexobarbital sleeping time, minutes	101 ±5	48[2] ±4	24[2] ±1

[1] Rats were pretreated with Aroclors 1016 or 1254, dissolved in corn oil, at a dosage of 25 mg/kg/day for 6 days. Controls received corn oil only. Each value represents mean ± S.E. for 5 rats.

[2] Values significantly different from the respective control values ($P<0.05$).

[3] No loss of righting reflex occurred in any of the 5 rats.

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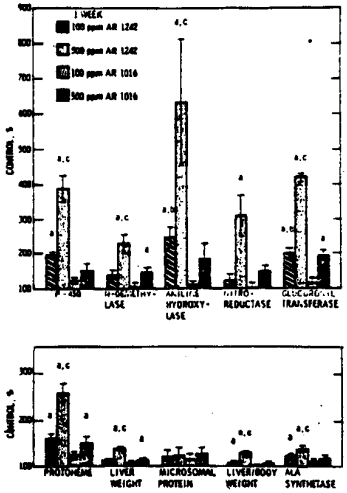
the experiment of Iverson et al. (243, Table III.10.5) probably results from the more extended dosage regime in the latter experiment, and emphasizes the complexity of the effects under review.

Goldstein et al. (245) compared the responses of female rats to Aroclors 1016 and 1242 after feeding at 100 and 500 ppm. After one week of feeding there were marked differences in response: Aroclor 1242 had markedly increased liver weight and all drug metabolizing systems tested including cytochrome P-450, N-demethylase, nitroreductase, aniline hydroxylase, and glucuronyl transferase, whereas Aroclor 1016 had produced only minimal effects (Figure III.10.7). After six months' exposure, however, the effects of the two mixtures were much more similar (Figure III.10.8). Thus the action of Aroclor 1016 on female rats appears to involve a time lag. It should be noted also that Goldstein et al. tested only female rats, whereas Iverson et al. (243) found that males were more sensitive to Aroclor 1016. Although in some experiments Aroclor 1016 had less pronounced effects on hepatic enzyme systems than Aroclor 1242, its effects thus appear similar after prolonged exposure.

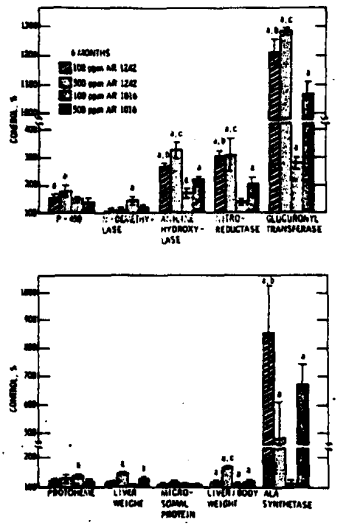
III.10.6. Enzyme induction by PCDFs.

No studies appear to have been reported of the activity of PCDFs in inducing hepatic microsomal enzymes in mammals. In chickens, 2,3,7,8-TCDF is as potent as 2,3,7,8-TCDD in inducing aryl hydrocarbon hydroxylases in the embryo (36), but it is much less active in inducing cytochrome P-450 in the hatched chick (146, 147).



. Effect of Aroclors 1016 and 1242 on cytochrome P-450, drug-metabolizing enzymes, protoheme, liver weight, liver microsomal protein, and ALA synthetase activity at 1 wk. Groups of six female rats were fed 100 or 500 ppm of Aroclors 1016 or 1242 and sacrificed at 1 wk. Values represent means ± SE. a—Significantly greater than controls at $p < 0.05$. b—100 ppm Aroclor 1242 significantly greater than 100 ppm Aroclor 1016, $p < 0.05$. c—500 ppm Aroclor 1242 significantly greater than 500 ppm Aroclor 1016, $p < 0.05$.

Figure III.10.7 (from ref. 245)



Effect of Aroclors 1016 and 1242 on cytochrome P-450, drug-metabolizing enzymes, protoheme, liver weight, liver microsomal protein, and ALA synthetase activity at 6 mo. Groups of six female rats fed 100 or 500 ppm of Aroclors 1016 and 1242 were sacrificed at 6 mo. Values represent means ± SE. a—Significantly greater than controls at $p < 0.05$. b—100 ppm Aroclor 1242 significantly greater than 100 ppm Aroclor 1016, $p < 0.05$. c—500 ppm Aroclor 1242 significantly greater than 500 ppm Aroclor 1016, $p < 0.05$.

Figure III.10.8 (from ref. 245)

III.10.7. Synergistic effects on enzyme induction.

Araki and Tanaka (246) reported significant synergistic effects between PCBs, DDT and BHC (HCH) in inducing hepatic microsomal enzymes in vivo. Using hexobarbital sleeping time as the indicator of enzyme activity, they found that the activities of DDT and β-HCH were about one-eighth of those of the same dose of PCBs (KC-400). When a small dose of DDT or β-HCH was combined with a very small dose of PCBs (each about one-quarter of the minimal effective dose when tested singly) the inducing activity was strongly potentiated.

III.10.8. Structural changes in liver.

Structural changes in liver cells, in addition to proliferated smooth endoplasmic raticulum, include: increase in lipid droplets within the cytoplasm, the formation of numerous multi-layered concentric membrane arrays, increase in numbers of microbodies and lysosomes within cells, and reduction of rough endoplasmic reticulum.

Microscopically, enlargement of individual hepatocytes was apparent. Present in the abundant cytoplasm of these cells were numerous small fat vacuoles, proliferated endoplasmic raticulum and large concentric membrane arrays (CMA). The CMAs encircled lipid droplets and other cytoplasmic organelles. There was a moderate increase in lysosomes. Other organelles, including the nucleus, were normal in appearance (188).

The CMAs may represent a means by which the hepatic cells are able to incorporate more membranes in a given area, thereby

enhancing metabolic activity of the cell. CMAs were observed in rats after three weeks on a PCB-containing diet. The arrays increased in size and complexity in direct relationship to the time the rats stayed on the experimental diet (188).

Several other liver lesions have been observed after exposure to PCBs. Dietary exposure (500 ppm) of Sherman rats to Aroclor 1254 for 6 to 8 months produced extensive areas of adenofibrosis in the liver. Fatty metamorphosis, accumulation of brown pigment in hepatic macrophages and Kupffer cells are also seen (187, 189, 208; Tables III.9.4, III.9.5). Adenofibrosis is firm and grayish white, with a pitted or granular surface. On microscopic examination it consists of fibrosis and proliferated, atypical glandular epithelium that forms ducts and cysts. The cysts usually contain mucus or cellular debris (189). Some clusters of atypical glandular cells resemble pancreatic tissue in appearance and staining properties (247). The relation of these and other liver lesions to the development of neoplasia is discussed below under Carcinogenesis (Section III.14).

Nishizumi reported some variation in size of nucleus of mouse liver cells after 17 weeks of oral administration of PCBs (1.5 mg/day). He also noted enlargement of Kupffer's cells in livers of PCB-treated mice and monkeys (210).

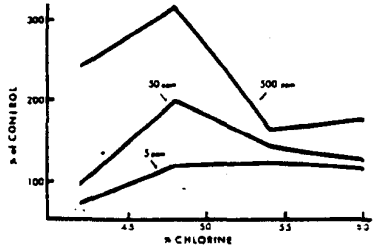
III.10.9. Changes in biochemical composition of the liver.

Biochemical data obtained from liver homogenates of PCB-treated rats clearly indicates hypertrophy of the liver. As a result of membrane proliferation and more numerous ribosomes, there was an

increase in protein content and RNA levels. Increased protein content of liver homogenate indicates more protein containing membranes within the cells. More ribosomes and the related increase in RNA would be necessary for production and maintenance of the elaborated membrane systems. These ribosomes are apparently not attached to the endoplasmic reticulum. A relative decrease in DNA, when expressed in relation to total liver weight, further signifies the proliferated state of the cytoplasmic components. There was no appreciable change in total DNA of liver or in amount of DNA per cell (188).

Changes in biochemical composition of the liver after exposure to PCBs include a significant increase in total lipid content (182). Triglyceride content of the liver increased with increased dosage of PCBs (from 5 to 500 ppm) in the diet (219). This increase was greater with Aroclor 1248 than with 1242, 1254 or 1260 (Figure III.10.9). The ratio of phospholipids to protein increased and the ratio of cholesterol to protein increased (221). The increase in liver lipid following PCB exposure is due in part to an increase in lipid synthesis and in part to a decrease in transport from the liver (248). Marked increases in liver lipids have also been noted in treated rabbits (256).

Allen and Abrahamson (188) observed compositional changes in liver microsomes of PCB-treated rats. Increase in protein content of microsomes was apparent soon after ingestion of PCBs and persisted throughout the four-week examination period. The levels of



Effect of dose and chlorine content of Aroclors on hepatic triglyceride concentrations in rats after 4 wk. Each point represents the mean of 3 replicates. With one exception all SD values were less than 10% of their respective means (SD of the 5 ppm dose of Aroclor 1248 was 18% of its mean).

Figure III.10.9 (from ref. 219)

phospholipids also increased moderately. There was a decided decrease in cholesterol and RNA in all experimental liver microsomes.

The increase in microsomal protein further substantiates the proliferated state of the endoplasmic reticulum since there would be more membranes per gram of liver. The decrease in microsomal cholesterol and the moderate increase in phospholipid content were, however, unexpected since proliferation of endoplasmic reticulum is usually accompanied by increases in protein, phospholipid and cholesterol at approximately the same rate. The decrease in microsomes is compatible with a proliferated state of the smooth endoplasmic reticulum. The increase in microsomal protein is related to an enhanced activity of the microsomal enzymes (188).

Bitman et al. (166) observed a very striking decrease of about 50% in liver concentration and content of vitamin A in rats fed 100 ppm Aroclor 1242 for 2 months. A decrease in liver storage of vitamin A in rats is also a symptom of repeated exposure to other chlorinated hydrocarbons (166, 5, 174).

III.11. Induction of Porphyria.

Porphyria cutanea tarda in humans is an acquired defect in hepatic porphyrin metabolism, characterized by uroporphorinuria (excretion of porphyrins in the urine), photosensitivity as manifested by blisters, and mechanical fragility of the skin (5). The hepatic porphyria which is responsible for the increase in porphyrins and for the skin photosensitivity can be produced experimentally by a number of drugs and chemicals which have the ability to stimulate activity

of the initial enzyme in the heme synthesis, δ-aminolevulinic acid (ALA)-synthetase (249: see Figure III.11.1).

PCBs produce hepatic porphyria in animals. Goldstein et al. (250) observed hepatic porphyria in Sherman rats exposed to Aroclor 1254 in the diet for at least seven months. The porphyria resembled human porphyria cutanea tarda. When female rats were fed 100 ppm Aroclor 1254 in the diet, they developed hepatic porphyria in two to four months. At this time, the urinary porphyrin excretion increased rapidly. Uroporphyin excretion was elevated to a maximum of 540-fold, coproporphyrin, porphobilinogen 50-fold and ALA 18-fold. Massive amounts of 8- and 7-carboxyporphyrins accumulated in the liver. The major porphyrins excreted in the urine of PCB-treated rats were 8-carboxyporphyrin (73%) and 7-carboxyporphyrin (16%). Substantial increase in amounts of 5- and 6-carboxylic porphyrins were also observed. Excretion of coproporphyrin increased 10-fold while excretion of uroporphyrin increased 1400-fold. In control rat urines, coproporphyrin made up 81% of the porphyrin fraction. Porphyrin concentrations were high in livers of PCB-dosed rats but only a trace of porphyrin was found in liver samples in control rats.

The tremendous accumulation of uroporphyrins in the liver and urine of rats fed Aroclor 1254 suggests that PCBs may affect uroporphyrin formation or utilization. Hemosiderin has been found in the liver of rats treated with Aroclor 1254 (187). A partial block in heme synthesis may be involved in PCB-induced porphyria.

Induction of porphyria by PCBs is dependent on protein synthesis, endogenous iron, and perhaps cytochrome P-450 (251). There

The porphyrin haem biosynthetic pathway.

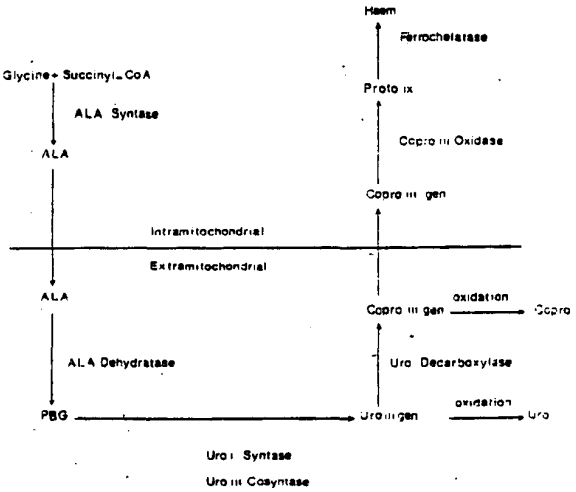
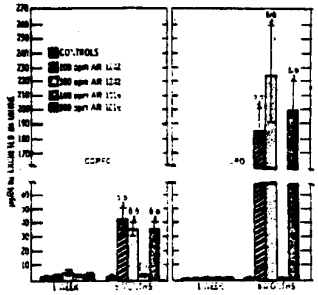


Figure III.11.1 (from ref. 248)

is some evidence that the active agent is an unstable metabolite, perhaps a hydroxylated chlorobiphenyl (251).

Aroclors 1016 and 1242 also induce porphyria in rats (243, 245). After dosing at 10 and 100 mg/kg/day for 21 days, both mixtures caused a significant accumulation of porphyrins in both sexes, but Aroclor 1242 was nearly twice as effective at 100 mg/kg and produced a significant effect at the lower dose (Tables III.10.5, III.10.6). After feeding at 100 and 500 ppm, both Aroclors induced porphyrins in liver and urine (Figure III.11.2, Table III.11.1), but Aroclor 1016 did so only at the higher dose. Induction of ALA-synthetase followed the same pattern (Figure III.10.8).

Rabbits developed symptoms of porphyria after application of Clophen A60, Phenoclor DP6 and Aroclor 1260 to their back skin (204). Increased fecal excretion of coproporphyrin and protoporphyrin was observed in rabbits with a chromatographic fraction of the PCB mixtures which would contain any dibenzofurans present. Hence Vos suggested that porphyria is caused by PCBs themselves and not by PCDFs (128, 204). This has been supported by the recent work of Goldstein et al. (146, 193), who found that mice fed 3,4,5,3',4',5'-hexaCB developed porphyria, but those dosed with 2,3,7,8-TCDF did not. In the same experiment 2,3,7,8-TCDD induced porphyria in mice (146), but TCDD is not a potent inducer of ALA-synthetase in other species of mammals (202, 252).



Effects of Aroclors 1016 and 1242 on urinary coproporphyrin and uroporphyrin excretion. Treatment is described in Fig. 3. Values represent means ± SE. The numbers above the bars represent the proportion of animals whose individual values were outside the 99% confidence limits of the controls at $p < 0.01$. a—Significantly different from controls, $p < 0.01$.

Figure III.11.2 (from ref. 245)

Table III.11.1 (from ref. 245)

EFFECT OF AROCLORS 1016 AND 1242 ON LIVER PORPHYRINS IN THE RAT[a]

Treatment	1 Week		6 Months	
	7-COOH	8-COOH	7-COOH	8-COOH
Control	Trace[b]	Trace[b]	Trace[b]	Trace[b]
100 ppm Aroclor 1242	Trace[b]	Trace[b]	164 ± 27	534 ± 61
500 ppm Aroclor 1242	Trace[b]	Trace[b]	227 ± 59	669 ± 146
100 ppm Aroclor 1016	Trace[b]	Trace[b]	Trace[b]	Trace[b]
500 ppm Aroclor 1016	Trace[b]	Trace[b]	89 ± 23	343 ± 65

[a] Porphyrins were methylated and analyzed by thin-layer chromatography. Female rats were treated as described in Fig. 1. Results are given as the mean ± SE.
[b] <1 μg/g.

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III.12. Miscellaneous Biochemical Effects

III.12.1. Hypertriglyceridemia

In addition to the increases in liver lipids noted above in Section III.10.8, PCBs have been shown to cause increases in serum lipids in rats (181, 253) and in rabbits (254, 255). The doses used to show these effects were quite high (100-300 mg/kg/day in rats, 10-100 mg/kg/day in rabbits).

III.12.2. Effects on the adrenal gland

Wassermann et al. (257) observed a rise in the activity of the zona fasciculata of the adrenal gland in PCB-treated rats. After ten weeks of receiving 250 ppm of Aroclor 1221 in their drinking water, rats had a mean corticosterone plasma level twice as high as control rats. Morphological changes in the zona fasciculata of the adrenal gland also indicated increased activity, corroborating the biochemical data.

III.12.3. Effects on thyroxine.

Hepatic conjugation of thyroxine to glucuronic acid and excretion in the bile of thyroxine-glucuronide is a major route of thyroxine metabolism. Thyroxine-glucuronide formation is rate limiting for biliary thyroxine excretion.

The effects of PCB on thyroxine metabolism resemble the effects of such polycyclic aromatic hydrocarbon (PAH) enzyme inducers as 3, 4-benzo(a)pyrene and 3-methylcholanthrene. The polycyclic hydrocarbon type of hepatic microsomal enzyme inducer enhances biliary

thyroxine-glucuronide formation (258). After daily treatment of rats with 25 mg/kg body weight of Aroclor 1254 for four days, biliary thyroxine excretion increased 4- to 5-fold. The ratio of iodine in bile to iodine in plasma was elevated tremendously. The rate of clearance of thyroxine from the blood through biliary excretion was greatly increased by PCB treatment. In PCB-treated animals, the proportion of free thyroxine in bile was reduced while the proportion of thyroxine in its metabolized form (thyroxine-glucuronide) was increased.

In PCB-treated rats, serum protein-bound iodine concentrations were less than half of normal. This may be attributed to enhanced biliary excretion of thyroxine and also to inhibition of thyroid hormone binding to plasma proteins. PCB, administered *in vivo* or added *in vitro* to rat serum, reduced binding of thyroid hormone to serum protein. This inhibition of plasma protein-binding of thyroxine may, in turn, contribute to the enhancement of biliary thyroxine excretion induced by PCB (258).

PCBs increased thyroid function, as shown by elevated uptake (258). For a review of effects of PCBs on the thyroid, see Jefferies (174).

III.12.4. Hematological effects.

Changes in blood composition and bone marrow histology have been observed in animals after exposure to PCBs.

Allen *et al.* (195) found that hematological changes developed gradually in rhesus monkeys fed diets containing 300 ppm Aroclor 1248.

Over three months, there was a decrease in hemoglobin of approximately 2 g/100 ml and a decrease in hematocrit from 40 to 33%. No major modifications of total white cell count were noted. However, there was a gradual decrease in the number of lymphocytes and a concomitant increase in neutrophils. There was also a decrease in total serum protein of 1.5 - 2 g/100 ml. A gradual shift in albumin/globulin ratio of serum protein also occurred. Allen et al. (215) speculate that PCBs may have an inhibiting effect on the red-blood-cell-forming tissue of bone marrow.

Bruckner et al. (259) found some evidence of anemia in rats given intraperitoneal injections of Aroclor 1242. Hematocrit, erythrocyte count, erythrocyte diameter, mean corpuscular hemoglobin concentration, and total blood hemoglobin concentration were all somewhat lower in PCB-dosed animals than in controls. An elevation in the leukocyte count was noted in the blood of PCB-dosed rats, with a large increase in the proportion of circulating neutrophils. Increased leukocyte counts were a consistent finding also in dogs chronically exposed to PCBs (212).

Other changes in blood of PCB-dosed animals observed by Bruckner et al. (259) include an elevation of serum iron and a decrease in both plasma corticosteroid and glucose concentration. Blood urea nitrogen concentration and serum bilirubin values were unchanged. The elevated serum iron and the decrease in hemoglobin concentration suggest a decreased incorporation of iron into protoporphyrin. Bruckner et al. (259) decided that the mild reduction in

erythrocyte count in PCB-dosed animals was not due to hemolytic action since they found identical bilirubin values in serum of PCB-dosed and control rats. No alteration in osmotic fragility of erythrocytes was observed.

III.12.5. Enzyme inhibition.

PCBs have an inhibitory effect on some enzymes. Hendrickson and Bowden (260) demonstrated *in vitro* competitive inhibition by PCBs of lactic dehydrogenase in rabbit muscle. Lactic dehydrogenase is the terminal enzyme of anaerobic glycolysis.

Sharp *et al.* (261) showed that beef brain and rabbit kidney ($Na^+ + K^+$) - ATPases were inhibited by PCBs. The ($Na^+ + K^+$)ATPase is generally thought to comprise the membranal sodium pumping system. The extent of enzyme inactivation increased as the degree of chlorination increased (262). Sharp *et al.* (261) obtained experimental evidence to support the hypothesis that PCBs inactivate membrane ($Na^+ + K^+$)ATPases by interfering with the stabilizing function of acidic phospholipids. Similar effects have been reported on ATPases in fish and have been linked with disruption of osmoregulation (127).

III.13. Immunosuppressive Effects

The ability of PCBs to inhibit immune responses has been demonstrated in several mammalian species. Following dermal exposure of rabbits to PCBs, Vos and Beems (204) observed atrophy of the thymus and a reduction in the number of germinal centers in spleen and lymph nodes. The thymus, spleen and lymph nodes are all lymphatic structures vital to the body's immune defense system. The germinal centers in spleen and lymph nodes are the principal productive field of lymphocytes. The thymus secretes a hormone that enables lymphocytes to develop into plasma cells which can synthesize antibodies against foreign proteins. Vos and Beems (204) also observed a reduction in total white cell counts in rabbits whose skin had been painted with PCBs. These observations suggest immunosuppressive action of PCBs.

Vos and de Roij (263, 129) reported a function test of the immunological system on three groups of twelve female albino guinea pigs fed 0, 10 and 50 ppm Aroclor 1260 for eight weeks. Tetanus toxoid injection was used to test the humoral immune response. They observed a significantly reduced number of γ-globulin-containing cells in lymph nodes of PCB-fed guinea pigs stimulated with tetanus toxoid, together with a significantly decreased γ-globulin level in the serum of toxoid-stimulated animals fed 10 ppm PCB. These observations demonstrated that some immunosuppression is produced by PCB-feeding.

Vos and van Driel-Grootenhuis (264) investigated cell-mediated immunity as well as the humoral immune response in guinea pigs. A

suppression of the humoral immunity, after stimulation with one dose of tetanus toxoid was found at the 50 ppm level. The antitoxin levels were significantly decreased. The number of antitoxin-producing cells in skin reactions after tuberculination was the parameter of cell-mediated immunity. This test indicated a significant reduction in cell-mediated immunity at the 50 ppm level of PCBs in the diet.

Major affects on the lymphoid system due to the suppressive activity of PCBs included strongly reduced weights of the thymus, lymph node and, to a lesser extent, of the spleen (128, 264). Atrophy of the thymus in newborn rodents reduced the capacity of these animals to produce serum antibodies, resulting in wasting disease (cachexia). Such animals are then incapable of rejecting foreign skin grafts (5). Cachexia and depletion of the lymphoid system were observed in PCB-fed guinea pigs (264).

Koller and Thigpen (265) compared effects of Aroclors 1221, 1242 and 1254 on the humoral antibody response of rabbits exposed to pseudo-rabies virus. Rabbits fed all three PCB formulations had significantly lower serum-neutralizing antibody titers to pseudo-rabies virus than did rabbits who were not given PCB formulations. The serum titers from rabbits given PCBs were approximately one-half those of controls. Aroclor 1242 produced the lowest titers, succeeded by Aroclor 1254 and then Aroclor 1221.

Atrophy of the thymus and/or the spleen has been reported in several other species treated with PCBs, including chickens (146, 147, 70), mice (146, 147), monkeys (197) and pigs (216). Atrophy

of the thymus and spleen are also striking effects of TCDF in chickens, guinea pigs and mice (70, 71, 146).

Immunosuppressive effects of PCBs may have been involved in several instances in which PCB-treated animals appeared more susceptible to disease than controls. These cases included increased susceptibility of piglets to septicemia (216), of ducks to viral hepatitis (178) and of fish to fungal disease (111).

Another potentially serious consequence of immunosuppression is an increased susceptibility to carcinogens, since suppression of immune response may make an animal more susceptible to establishment of neoplastic cell lines and development of malignant tumors.

III.14. Carcinogenic and Co-Carcinogenic Effects

Studies of carcinogenic and co-carcinogenic effects of PCBs are reviewed in detail in Appendix D and only a brief summary is included here.

In a long-term study by Kimbrough et al. (266), Sherman rats fed 100 ppm Aroclor 1260 in the diet for 21 months developed hepatocellular carcinomas (26/184) and neoplastic nodules (144/184) in the liver, whereas 173 control animals developed only one carcinoma and no neoplastic nodules. The terminology used to describe the liver lesions is that developed at a recent workshop of experts in liver pathology (269), where some of the slides from the PCB-treated animals were reviewed. The study reported in ref. 266 followed a sequence of shorter-term studies in which the earlier stages of development of liver lesions in PCB-treated rats had been described (see Section III. 10.a); in one of these studies, liver nodules had been reported in the second generation of rats exposed to 20 ppm Aroclor 1254 at an age of only 330 days (179). Other significant findings in the livers of treated rats were adenofibrosis (cholangiofibrosis) (189) and abnormal pancreatic-type tissues (247). The significance of these findings is not clear. Adenofibrosis has been reported in the livers of rats fed with carcinogens (268); some experts believe that they have the potential to develop into cholangiocarcinomas (283), although an earlier review concluded that they were not convincingly pre-cancerous (284).

In another long-term study (207), groups of Charles River rats were fed with 1, 10, and 100 ppm Aroclor 1242, 1254, and 1260 for 24

months. Although the initial raport indicated only mild liver lesions (207), a more recent review (68) reports tha occurrence of lesions classified as nodular hyperplasia, hepatomas and cholangiohepatomas in 44/74 of the rats surviving near to the end of the experiment in the groups fed 100 ppm of all three Aroclors. Although there are some terminological problems and findings have not been adequately reported, these results appear consistent with those of Kimbrough et al. and suggest an oncogenic potential for all three Aroclors. There was also an excess incidence of pituitary tumors (chromophobe adenomas) in all treated groups in this experiment.

Other studies reviewed in Appendix D were conducted for much shorter periods and generally at higher dose levels; they constitute little more than preliminary screening tests. Kanechlors KC-500, KC-400, KC-300 induced nodular hyperplasia and cholangiofibrosis, but not hepatocellular carcinomas, in rats within 28-52 weeks (190). KC-400 induced multiple adenomatous nodules in the livers of rats within 400 days (271). Aroclor 1254 induced adanofibrosis and hepatomas in mice fed for only 6-11 months (191). KC-500 induced nodular hyperplasia and hepatocellular carcinoma in mice within 32 weeks, whereas KC-400 and KC-300 did not do so within the same period (192).

These six studies are consistent in showing that PCB mixtures with 42-60% chlorine content induce neoplastic and pre-neoplastic lesions in the livers of rats and mice, and that this induction is very rapid at high dietary levels (250-500 ppm). Because most of the studies have been terminated early, the full development of the lesions

has not yet been studied. No long-term feeding studies have yet been reported which have failed to show some evidence of neoplastic change: the longest such study was terminated after 12 months (69).

Among co-carcinogenic studies conducted in vivo, KC-500 markedly promoted or accelerated the hepatocarcinogenicity of α-BHC and β-BHC in mice (192). However, KC-400 did not promote the development of cervical carcinomas in mice induced by methylcholanthrene (272), and KC-500 inhibited or delayed the hepatocarcinogenicity of three other carcinogens in rats (273).

Numerous studies have shown that PCBs, by stimulating the cytochrome P-450 dependent mixed function oxidase system in the mammalian liver, can activate secondary carcinogens and mutagens or de-activate primary carcinogens and mutagens. Pretreatment with PCBs markedly increases liver injury by vinyl chloride, a known human liver carcinogen, but decreases injury by 1,1-dichloroethylene (274). PCBs promote the metabolism of dimethylnitrosamine to an active mutagen (281, 282), but de-activate a primary carcinogen (MNNG). PCBs are exceptionally efficient stimulators of the mixed function oxidase system and are now used routinely to activate suspect carcinogens in bacterial mutagenesis bioassays (276, 280).

PCBs are known to stimulate the mixed function oxidase system at extremely low doses (219) and their action in doing so is potentiated by other environmental pollutants such as BHC and DDT (246).

In addition to their direct carcinogenic effects and co-carcinogenic effects, the immunosuppressive effects of PCBs are also expected to be important in potentiating the action of other carcinogens.

III.15. Mutagenic and Teratogenic Effects

Green et al. (285) found no evidence of cytogenetic effects in bone marrow or spermatogonial cells of rats after treatment with single or multiple doses of Aroclors 1242 and 1254. There was a significant decline in the rate of cell division in rats treated with multiple doses (150-500 mg/kg/day for 4-5 days). Calandra (68) reported that a dominant lethal mutagenic study with Aroclors 1242, 1254, and 1260 in mice had given negative results, but details were not presented.

Villeneuve et al. (218) reported that single oral doses of 12.5, 25 and 50 mg/kg Aroclor 1254 administered to pregnant rabbits were fetotoxic, but did not induce teratogenic effects. Linder et al. (179) reported that Aroclors 1254 and 1260 had minor fetotoxic effects when administered at 100 mg/kg/day to female rats on days 7-15 of pregnancy; Aroclor 1254 also caused substantial mortality in pups prior to weaning. The pups were described as "grossly normal" but apparently were not autopsied.

Teratogenic effects and mutagenic effects of Aroclor mixtures have been reported in birds (141, 154, 155, 156, 157, 158, 159, 161). By analogy with PCDDs, PCDFs might be expected to be mutagenic and/or teratogenic. Hence it is regrettable that the studies in mammals have been reported incompletely. Except for that of Green et al. (285), none of the studies listed above can be regarded as satisfactorily negative.

III. 16 Effects in Humans

III. 16.1 Chloracne

Chloracne is an occupational disease associated with occupational exposure to PCBs, poly-chlorinated naphthalenes (PCNs), chlorinated phenols, 2,4,5-T, PCDFs, and perhaps polychlorinated diphenyl ethers. The etiology of the disease has been reviewed by Schulz (286), Crow (287), and Kimbrough (5).

Exposed workers develop small dermal cysts, pustules, and comedos. These are most commonly found on the face and ears, although other areas are commonly effected, including the abdomen, back, thighs, forearms, buttocks, and scrotum. This widespread distribution has been ascribed to direct contact of the affected parts with contaminated clothing. Melanosis and secondary inflammation may occur also. Systemic effects have also been stated to occur in several cases, perhaps due to inhalation of toxic vapors. These systemic effects include nausea, lassitude, anorexia, digestive disturbance, edema of the face and hands, abdominal pain, vomiting, burning and soreness of the eyes, impotence and hematuria (2,5). Deaths due to liver damage have also been reported in association with exposure to PCNs (2,5). Hepatic porphyria has been associated with exposure to 2,4,5-T (5), but Crow has suggested that these cases might in fact be attributable to exposure to chlorinated benzenes used in the same plant (287).

The onset of chloracne following initial exposure is usually quite slow. After occupational exposure to PCBs and PCNs, the latent period is typically about 7 months. The course of the disease is protracted. Chloracne usually persists for several months following removal from the source and may last up to four years (2).

The extent to which PCBs, and PCDFs, and other chemicals are responsible for chloracne is still not fully clear. PCDFs are said to be particularly toxic and acneigenic and to have been abandoned by industry for that reason (287). The symptoms arising from exposure to the other chemicals named above are said to be generally similar, except for the liver effects associated with PCNs. In the case of 2,4,5-trichlorophenol, Kimmig and Schulz, using an animal model, traced the acneigenicity primarily to contaminants, which they identified as PCDDs, especially 2,3,7,8-TCDD (288). Chloracne has been induced in human volunteers by skin application of TCDD and commercial PCNs (5, 286). However, Vos and Beems (204) reported that commercial PCNs are contaminated with PCDFs, as a result of the contamination of commercial naphthalene with dibenzofuran. As pointed out in Section II.5.5 above, commercial PCBs also are now known to contain trace quantities of PCDFs. Hence the relative roles of PCBs, PCNs, and PCDFs in causing chloracne are confused.

According to Crow, chloracne has been associated primarily with mixtures containing penta- and hexachloronaphthalene, and tetra, penta- and hexa-chlorobiphenyl. This suggests a structure-toxicity relationship parallel to that of the CDDs (148). Chloracne is now reported much less frequently than prior to 1944, and only one recent outbreak associated with manufacture of PCBs has been reported (5, 289). It is not clear whether the decline in cases associated with PCBs reflects improvements in industrial hygiene, reduction in PCDF contamination, or other factors. Epidemiological studies have recently been started (290).

No long-term follow-up studies of occupationally exposed workers have yet been reported, so there is no information on the carcinogenicity of PCBs.

MONS 064413

III.16.2 Yusho

"Yusho" is a disease reported in Japan late in 1968 and traced to consumption of rice-oil contaminated with PCBs from a leaking heat-exchanger on 5-6 February 1968. Extensive reports on Yusho have been published by Japanese investigators and several summaries in English are available (35, 291-293). At least 1,291 persons were affected by the disease and descriptive epidemiology has been presented for 325 cases (291).

The contaminated rice-oil is reported to have contained about 1,000 ppm of PCBs (Kanechlor KC-400) and is now known to have contained also about 5 ppm of a mixture of CDFs (34, 35, ; Tables II.5.3, III.16.1). Thus the Yusho episode involved proportionately much greater exposure to CDFs than is normal for occupational exposure to PCBs or likely for environmental exposure to PCBs. This provides some interesting information about the effects of PCDFs on humans, but limits the value of the Yusho episode as a predictor of the effects of environmental exposure to PCBs.

A rough dose-response relationship was constructed relating severity of symptoms to estimated consumption of the oil (Table III.16.1). Severe cases were induced by consumption of less than 700 mg of PCBs (and less than 3.6 mg of PCDFs). According to Kuratsune (291) the smallest dose causing overt symptoms was about 500 mg of PCBs ingested over approximately 50 days by an individual weighing about 50 kg (i.e. about 200 µg/kg/day PCBs).

However, these figures are probably too high by a factor of 2-3, since they were based on an estimate of 2,000-3,000 ppm for the concentration of PCNs in the Yusho oil, whereas the most recent measurements place the average concentration at only 900-1,000 ppm (Table III.16.2).

Table III.16.1 (from ref. 291)

Relation between the amount of the K rice oil used by patients and their clinical severity.

Amount of oil	Non-affected		Light cases		Severe cases		Total	
	No.	%	No.	%	No.	%	No.	%
Less than 720 ml	10	(12.0)	39	(49.0)	31	(39.0)	80	(100.0)
720–1,440 ml	0	(0.0)	14	(31.0)	31	(69.0)	45	(100.0)
More than 1,440 ml	0	(0.0)	3	(14.0)	18	(86.0)	21	(100.0)

MONS 064415

Table 3: PCDF's and PCB's in Kanechlors and Yusho oil.

Sample		PCDF's (ppm)		PCB's (ppm)	
		Peak height method	Perchlorination method	Peak height method	Perchlorination method
Kanechlor	300	1	1.5	-	-
	400	18	16.6	-	-
	500	4	2.5	-	-
	600	5	2.7	-	-
Yusho oil	A	5	4.4	830	870
	B	4	5.1	900	920
	C	5	5.2	1030	980

Table III.16.2 (from ref. 35)

MONS 064416

Hence the minimal dose for overt symptoms would have been about 70-100 ug/kg/day of PCBs, together with about 0.4 μg/kg/day of PCDFs. The most highly exposed individuals would probably have ingested more than 3 μg/kg/day of PCDFs (Table III.16.1), a dose that is comparable with the lethal dose of 2,3,7,8-TCDF in guinea pigs (71). However, it is not clear that any individuals actually died as a direct result of Yusho (291).*

As in the case of chloracne, the onset of symptoms was delayed: reported cases peaked 3-7 months after the contaminated oil was shipped (291, Table III.16.3). Table III.16.4 summarizes clinical findings on 180 patients studied in detail. The general clinical findings were similar to those in industrial chloracne, but the Yusho patients had several additional symptoms. Outstanding among these were hypersecretion of the Meibomian gland, and hyperpigmentation of the skin; the latter was associated with increased melanization. Porphyrins were not measured in Yusho victims, but other signs of porphyria such as photosensitivity were not reported. Liver damage apparently was not marked, but there was elevation of serum alkaline phosphatase in severe cases, and increase in smooth endoplasmic reticulum in biopsied material (292). Other characteristic findings in Yusho were elevated blood-serum triglycerides, increased excretion of 17-ketosteroids in the urine, respiratory distress with secondary infections (292), and peripheral neuropathy (Table III.16.4). Unfortunately parameters of the hepatic mixed function oxidase system do not appear to have been measured.

* One 25-year-old male admitted to hospital in June 1969 (one year after contracting Yusho) with severe abdominal pain and suspected perforated duodenal ulcer died from heart failure 3 weeks after operation (401).

Two Yusho victims who died in 1968-69 were autopsied. In addition to pathological skin changes, both had cystic dilatation, hyperplasia, and hyperkeratosis of the excretory duct of the esophageal gland. The 25-year-old male mentioned in the footnote to the previous page also had marked fibrinous pericarditis and multiple myocardial fibrosis with myofibrillar degeneration (401).

A number of live-born and at least 2 still-born infants were afflicted with Yusho (34, 291, 295). Their symptoms included hyperpigmentation of the skin, nails and gingivae, eye discharge, abnormally large fontanelles, swollen faces, and exophthalmos (259). The abnormal

Table III.16.3 (from ref. 291)

Distribution of patients with yusho diagnosed by January 20, 1969, by sex and month when symptoms appeared.

Month	Males		Females		Total	
	Number	Percent	Number	Percent	Number	Percent
Before 1968	2	1.3	2	1.2	4	1.2
1968						
January	0	0.0	0	0.0	0	0.0
February	4	2.5	6	3.6	10	3.1
March	8	5.1	12	7.2	20	6.2
April	15	9.5	10	6.0	25	7.7
May	18	11.4	15	9.0	33	10.2
June	23	14.6	33	19.8	56	17.2
July	27	17.1	32	19.2	59	18.1
August	34	21.5	30	18.0	64	19.7
September	17	10.8	12	7.2	29	8.9
October	8	5.1	14	8.4	22	6.8
November	1	0.6	1	0.6	2	0.6
December	1	0.6	0	0.0	1	0.3
Total	158	100.0	167	100.0	325	100.0

MONS 064419

Table III.16.4 (from ref. 291)

. Percent distribution of symptoms of Yusho reported by 189 patients examined before October 31, 1968.

Symptoms	Males (N = 89)	Females (N = 100)
Dark brown pigmentation of nails	83.1	75.0
Distinctive hair follicles	64.0	56.0
Increased sweating at palms	50.6	55.0
Acnelike skin eruptions	87.6	82.0
Red plaques on limbs	20.2	16.0
Itching	42.7	32.0
Pigmentation of skin	75.3	72.0
Swelling of limbs	20.2	41.0
Stiffened soles in feet and palms of hands	24.7	29.0
Pigmented mucous membrane	56.2	47.0
Increased eye discharge	55.8	53.0
Hyperemia of conjunctiva	70.8	71.0
Transient visual disturbance	56.2	55.0
Jaundice	11.2	11.0
Swelling of upper eyelids	71.9	74.0
Feeling of weakness	58.4	52.0
Numbness in limbs	32.6	39.0
Fever	16.9	19.0
Hearing difficulties	18.0	19.0
Spasm of limbs	7.9	8.0
Headache	30.3	39.0
Vomiting	23.6	28.0
Diarrhea	19.1	17.0

MONS 064420

pigmentation gradually faded but growth was normal, in contrast to that of children poisoned post-natally, whose growth was retarded.

One infant born before its mother was poisoned apparently contracted Yusho via breast-milk (296).

Recovery from Yusho has been very slow. Table III.16.5 shows that there was little significant improvement during the first two years. By 1974, the dermal and mucosal symptoms that had been most marked at the beginning of the episode had gradually improved, while symptoms such as general fatigue, poor appetite, inconstant abdominal pain, heavy headedness and headache, feeling of numbness and pain at the limbs, cough and expectoration of sputum, have become more prominent year by year (297: Table III.16.6). These are considered to reflect some internal disturbances (35).

Serum triglyceride levels have remained high in males, but have started to decline in females (Table III.16.7). Poisoned women continued to give birth to affected children at least until 1972 (35). However, liver function tests have not yet given evidence of serious liver lesions (35).

A preliminary tabulation of deaths among Yusho patients (Table III. 16.8) suggests the possibility of an excess of cancer, especially in the stomach and liver, but there are too few cases as yet to draw conclusions.

The scanty data available suggest that tissue levels of PCBs in Yusho victims declined rapidly in the first year after their poisoning and are now only a few times higher than the average in the Japanese population (Table III.16.9). However, dermatological symptoms and other signs, including serum triglyceride levels, are correlated with concentrations of PCBs in the blood (Table III.16.10).

Table III.16.5 (from ref. 291)

Prognosis of patients with Yusho.*

Grade of clinical severity**	Clinical conditions			
	Improved	Stational	Worsened	Total
0	13 (56.7)	6 (25.0)	2 (8.3)	24 (100.0)
1	30 (51.7)	16 (27.6)	12 (20.7)	58 (100.0)
2	23 (52.3)	16 (36.4)	5 (11.4)	44 (100.0)
3	12 (52.2)	10 (43.5)	1 (4.3)	23 (100.0)
4	0 (0)	10 (100.0)	0 (0)	10 (100.0)
Total	81 (50.9)	58 (36.5)	20 (12.6)	159 (100.0)

* Calculated from the figures reported by *Toshitani* and *Kitamura*.

** 0: Physical complaints without skin-lesions.
1: Pigmentation of the skin and the mucous membrane.
2: Comedo formation.
3: Acneform eruptions.
4: Extensive distribution of acneform eruptions.

MONS 064422

Table III.16.6 (from ref. 35)

Frequency of subjective symptoms complained by patients with Yusho from 1973 to 1974

Symptoms	Proportion[a] %
Fatigue	51.4
Headache	41.7
Phymata in articular region	8.3
Fever	2.8
Cough and sputum	56.9
Digestive disorder	40.3
Numbness of extremities	33.3
Menstrual disturbance	26.9 (7/26)

[a]Calculated by Kuratsune from original figures published by Koda and Masuda (ref. 4).

Results of followup study on serum triglyceride levels in patients with Yusho[A].

Patients			Triglyceride (mg/100ml) Mean ± S.D.					
Sex	Age	No.	1969	1970	1971	1972	1973	1974
Male	11 - 73	14	159 ± 57	166 ± 55	169 ± 60	174 ± 69	164 ± 68	160 ± 118
Female	7 - 59	26	155 ± 75	161 ± 70	155 ± 80	153 ± 63	129 ± 50[B]	111 ± 56[B]

A: Cited from a report by Okumura et al. (ref.19).
B: Significantly lower than in 1969, 1970, 1971, and 1972 ($p < 0.05$).

Table III.16.7 (from ref. 35)

MONS 064424

Table III.16.8 (from ref. 35)

Deaths seen among patients with Yusho[A].

Cause of death	Number
Malignant neoplasms	9
Stomach cancer	2
Stomach cancer + liver cancer	1
Liver cancer + liver cirrhosis	1[B]
Lung cancer	1
Lung tumor	1
Breast cancer	1
Malignant lymphoma	2
Cerebrovascular lesion	3
Amyloidosis	1[B]
Osteodystrophia fibrosa	1[B]
Myocardial degeneration + pericarditis	1[B]
Status thymicolymphaticus	1[B]
Liver cirrhosis	1
Suicide	1
Senility	1
Traffic accidents.	3
Total	22

A : Cited from a report by Urabe 1974 (ref.2).
B : Autopsied cases.

Table III.16.9 (from ref. 35)

PCB's concentration in tissues of patients with Yusho and other diseases.

Case	Time of death, operation	PCBs (ppm) Skin whole basis	Skin fat basis	Adipose tissue whole basis	Adipose tissue fat basis	Liver whole basis	Liver fat basis	Reference
Case 1 High school boy	Nov.1968			76 (Face) 13 (Abdomen)				
Case 2,3 Adult male, female	Nov.1968			32,46 (cheese-like substance from acneform eruptions)				10
Case 4 Boy, 13 yrs.	July 1969			1.3 (mesentery)	3.7	0.14	9.5	
Case 5 Male 25 yrs.	July 1969	1.2	8.7	2.8 (mesentery)	15.1	0.2	10.4	
Case 6 Male 73 yrs.	Nov.1969	1.0	4.4	3.8 (mesentery)	8.4	0.07	3.1	11,12
Case 7 Female 48 yrs.	Dec.1970	0.6	0.8	0.7 (mesentery)	0.9	0.07	1.3	
Case 8 Male 46 yrs.	May 1972	1.8	3.2	4.3 (mesentery)	6.5	0.08	8.4	
Case 9 Female 53 yrs.	Sept.1972			1.9 (subcutaneous)	2.9			
Case 10 Male 72 yrs.	April 1975			0.19 (mesentery)	0.4	0.04	3.0	14
National survey males and females 25-49 yrs.	1973	0.04-1.7 (N=54)		0.2-4 (N=47)	0.3-6.4 (N=48)	0.01-0.6 (N=51)	0.2-3.1 (N=30)	13

MONS 064426

Table III.16.10 (from ref. 35)

Prevalence of dermatological and other signs among patients with Yusho from April 1973 to March 1974, in connection with concentration and gaschromatographic pattern of PCB's in blood.

Signs		Prevalence (%)[C]			
		Group A (43 cases)	Group B (26 cases)	Group C (3 cases)	Total (72 cases)
Pigmentation	Skin	51.2[B]	0[B]	0	30.6
	Palpebra	72.1[B]	19.2[B]	0	50.0
	Gingiva	95.3[B]	57.7[B]	66.6	80.6
	Nail	74.4[B]	34.6[B]	0	56.9
Acneform eruption		34.9[B]	0[B]	0	20.8
Comedo		34.9	23.1	0	29.2
Infection of skin		32.6	11.5	0	23.6
Deformation of nail		65.1[A]	38.5[A]	0	52.8
Alopecia		0	3.8	0	1.4
Disorder in teeth		18.6	7.7	0	13.9
Hypersecretion of Meibomian gland		93.0	80.8	100.0	88.9
PCB's in blood	Conc. (ppb) Av.±S.D.	7.2±4.9	4.3±3.1	1.7±0.2	5.9±4.5
	Pattern	A[D]	B[D]	C[D]	

A : significant ($P < 0.05$) difference.

B : significant ($P < 0.01$) difference.

C : calculated by Kuratsume from figures published by Koda and Masuda (ref. 4).

D : "A" means the characteristic gaschromatographic pattern of PCB's remaining in the body of most patients with Yusho. "B" means gaschromatographic patterns somewhat similar to "A". "C" means patterns indistinguishable from those of normal persons.

MDNS 064427

An important new finding is that PCDFs have been retained in the tissues of Yusho victims, expecially in the liver where their ratio to PCBs is as high as 1:4 (Table III.16.11), versus 1:200 in the Yusho oil and 1:60,000 in the original Kanechlor mixture (Table III.16.2). The CDFs found in the tissues consist mainly of one penta- and one hexa-CDF: most of the isomers in the Yusho oil have not been retained (Figure III.16.1). The gas-chromatographic pattern of PCBs in Yusho victims also differs from that in ordinary persons (Figure III.16.2). Thus there appears to have been selective metabolism and/or excretion of certain CBs and CDFs in Yusho victims. The possibility that CDFs have been formed in the liver by metabolism should not be overlooked.

From the data available it is not possible to determine to what extent the persistence of symptoms in Yusho is due to tissue injury, persistence of PCBs in the body, and/or persistence of PCDFs in the body. The parallels between Yusho and chloracne are very close, and it is not clear to what extent the differences (such as the greater frequency of hyperpigmentation and eye discharge) are attributable to the high concentration of PCDFs in the Yusho incident.

III.16.3 Animal models for Yusho and chloracne

Yusho and chloracne are closely related to two diseases in domestic animals. "Chick edema disease" has been discussed in Section III.6.2: the incident of contamination which caused the Yusho episode in 1968 also caused an outbreak of chick edema disease (144). "X-disease" in cattle, characterized primarily by hyperkeratosis, but also by diarrhea, poor appetite and symptoms of the mucous membranes, has been associated with exposure to PCNs and PCBs (5, 298). In each case it is likely that PCDFs are responsible for some of the toxic effects (298, Section III.6.2).

Table ... PCB's and PCDF's in tissues of patients with Yusho and ordinary persons.

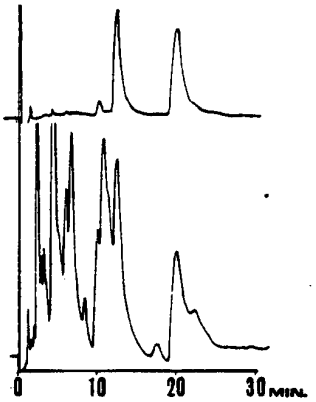
Subjects	Tissue	Case No.	Time of Death	PCBs (ppm) basis		PCDFs (ppm) basis		Ratio PCBs/PCDFs	
				Whole	Fat	Whole	Fat	Whole	Fat
Yusho patients	Adipose	1	1969	1.4	3.4	0.013	0.03	108	113
		2	1969	1.3	8.5	0.006	0.04	217	213
		3	1972	1.2	2.1	0.007	0.01	171	210
		Av.		1.3	4.7	0.009	0.03	144	157
	Liver	1	1969	0.05	4.7	0.025	2.3	2	2
		2	1969	0.06	5.6	0.010	1.1	6	5
		3	1972	0.03	3.5	0.003	0.3	10	12
		Av.		0.05	4.6	0.013	1.2	4	4
Ordinary persons	Adipose	1	1975	1.0	1.4	ND	ND		
		2	1975	0.4	0.7	ND	ND		
	Liver	1	1975	0.08	1.3	ND	ND		
		2	1975	0.02	1.0	ND	ND		

Table III.16.11 (from ref. 35)

MONS 064429

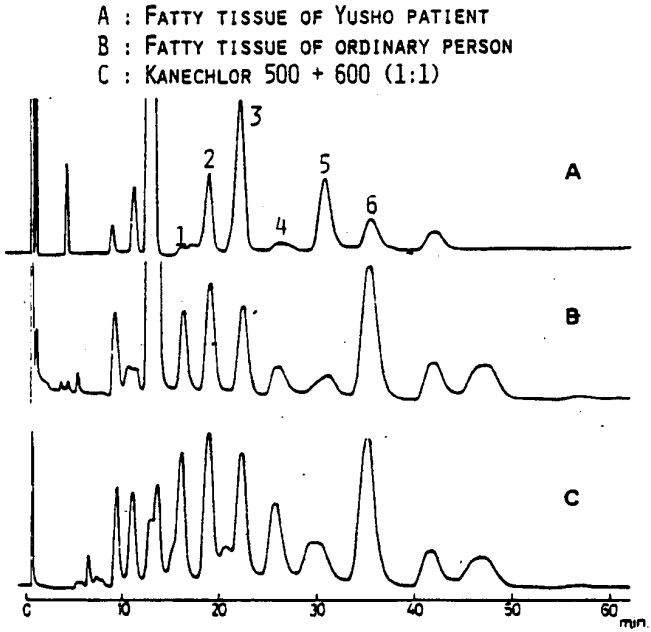
UPPER :
PCDF FRACTION FROM
LIVER OF YUSHO PATIENT.

LOWER :
PCDF FRACTION FROM
YUSHO OIL.



Gaschromatograms of PCDF fractions from liver of patient with Yusho and from "Yusho oil."

Figure III.16.1 (from ref. 35).



Gaschromatograms (ECD) of PCB's on SE-30.

Figure III.16.2 (from ref. 35)

Some traditional laboratory animals have proved to be poor models for Yusho and chloracne. Rodents in particular seem to be relatively resistant both to PCBs and to PCDFs (Sections III.6 - III.10 above) and some of the symptoms induced in rodents, such as hepatic porphyria, are not prominent in affected humans. However, hairless mice fed Yusho oil developed skin lesions, including hyperkeratosis of the hair follicle and sweat gland and cystic dilatation (298). These symptoms appeared more severe than those in mice exposed to Kanechlor KC-400 (210), suggesting a possible effect of PCDFs. However, mice were unaffected by doses of 2,3,7,8-TCDF as high as 6 mg/kg (71), 100 times higher than the cumulative doses of PCDFs ingested by Yusho victims. Rats also were affected by Yusho oil, showing hyperkeratosis of the epidermis and hair follicles, parakeratosis of epidermis, and hyperplasia and keratotic metaplasia of the ductal epithelium in the Meibomian glands of the eyelids (403). These symptoms parallel those of the Yusho victims, but have not been reported in other experiments with rats.

Rabbits have been used as a model for screening acneigenic agents (5, 129, 288). Rabbits are very sensitive to toxic effects of PCDFs (129) and application to rabbit skin causes chloracne-like symptoms (288). Vos and Beems (204) found that the rabbit skin test was a sensitive bioassay for PCDF contamination in PCB mixtures, but as discussed above a later experiment suggested that individual PCB isomers also have acneigenic action.

Recent work with rhesus monkeys suggests that this species is the best model for chloracne and Yusho (69, 194-198). The symptoms

found in rhesus monkeys and their offspring fed low doses of PCBs are quite similar to those found in humans. It is not yet clear whether the severe gastric ulceration found in monkeys (and to a lesser extent in other species, but not in rodents) has a parallel in humans, but the long persistence of digestive disturbances reported in victims of Yusho and chloracne suggests that it may do so. The occurrence of cystic dilatation and hyperplasia of the duct of the esophageal gland in two autopsied victims of Yusho (401) is a close but not exact parallel to the findings in monkeys.

III.16.4 The role of PCDFs in the toxicity of PCBs to humans

A key question in formulating criteria for human exposure to PCBs is the extent to which PCDFs or other contaminants may be responsible for the toxicity of commercial PCBs or environmental residues. Since many of the symptoms of chloracne and Yusho are known to be produced in humans (287) or animal models (205, 129, 299, 288) by PCDFs and PCDDs, it could be hypothesized that PCDFs are the primary or exclusive toxic agents. However, as pointed out in Section III.8 above, PCB mixtures with extremely low levels of PCDFs have caused chloracne in monkeys, and apparently pure chlorobiphenyl isomers have done so also (197). Hence it seems more probable that some chlorobiphenyls -- perhaps isomers with 3,4,3',4'-substitution or other structural similarity to 2,3,7,8-TCDF -- or their metabolites share the toxic properties of PCDFs. In any case, since PCDFs are present in commercial mixtures (Section II.5.5) and appear to be formed from them in the environment (Section II.8.3), in service (Section II.8.4) and by metabolism (Appendix E), it is difficult and probably academic to attempt to distinguish their effects completely in weighing the environmental hazards posed by PCBs.