Exhibit 9

(9)

Toxicities of PCBs to Fish and Environmental Residues

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Polychlorinated biphenyls (PCBs) have been found in fish and wildlife from many parts of the world at levels which may adversely affect aquatic organisms and interfere with pesticide residue analyses (1, 2, 3, 4). The environmental occurrence, uses, and present toxicological aspects of PCBs were recently reviewed by Peakall and Linder (5), Gustaffson (6), and Risebrough (7). PCBs have gas chromatographic characteristics similar to many organochlorine insecticides. Jensen (8) first identified unknown gas chromatographic peaks as PCBs in extracts of pike and an eagle which were analyzed for organochlorine insecticides.

The Monsanto Company is the sole manufacturer of PCBs in the United States (6) and markets eight formulations of chlorinated biphenyls under the trademarks Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. The latter two digits designate the percent chlorine of each formulation. Aroclor 1248 and 1254 are the materials produced in greatest quantities and are used as a dielectric fluid in capacitors and in closed-system heat exchangers (6). Aroclor 1242 is used as a hydraulic fluid, and Aroclor 1260, as a plasticizer. Chlorinated terphenyls are marketed under the trademark Aroclor 5442 and 5460. A mixture of bi- and terphenyls is designated Aroclor 4465. The Monsanto Company has restricted the sale of PCBs for uses in which disposal of the end products cannot be controlled, such as the use of PCBs as plasticizers (6).

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Chemical Composition and Analysis

PCBs occur in the aquatic environment as mixtures of chlorinated biphenyl isomers (10, 11). The biphenyl structure may be substituted with one to ten chlorine atoms, and over 200 compounds are possible (6). The isomer composition and chromatographic characteristics of each formulation have been described by Stalling and Huckins (12) and Bagley (13). The PCBs can cause serious interference in gas chromatographic (GC) determination of chlorinated insecticides (1). Analysis of PCBs is best accomplished by GC after separation of PCBs and pesticides during sample cleanup using silicic acid column cleanup (14). No standardized GLC method has been proposed for the analysis of mixtures of PCB formulations in environmental samples. The solubility of these formulations in water have not been precisely determined but is reported in the range of 100–1000 μg/l (9). Considerable research remains to be done on the structure of the isomers of each technical material and how environmental residues of various Aroclors are to be reported.

Environmental PCB Residues

Analyses of 40 fish (cross-check sample) from the 1970 National Pesticide Monitoring Program were made using sample preparation techniques which separated PCBs from pesticides and other industrial residues (Table 1). These analyses employed GC and gas chromatography-mass spectrometry (GC-MS) for confirmation. The samples were prepared using gel permeation chromatography (23), and PCBs were separated from the pesticides with silicic acid (14). Only

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Table 1. Composition of PCB residues in selected fish samples from the 1970 National Pesticide Residue Monitoring Program.

River	Location	Species	PCB residue as Aroclor type (μg/g whole body)				
			1232	1248	1254	1260	Total
Ohio	Cincinnati, O.	Carp	10.2	75	42	6.0	133
Ohio	Cincinnati, O.	White crappie	15.9	17	27	5.6	65
Ohio	Marietta, O.	Channel catfish	38	23	11	4.9	77
Ohio	Marietta, O.	Channel catfish	15.6	5.2	12.6	4.6	38
Yazoo	Redwood, Miss.	Smallmouth buffalo	72	—	1.4	—	73
Hudson	Poughkeepsie, N.Y.	Goldfish	8.6	173	32	—	213
Allegheny	Natrona, Pa.	Walleye	—	5.2	25	4.6	35
Delaware	Camden, N.J.	White perch	—	8.0	6.8	2.9	18
Cape Fear	Elizabeth Town, N.C.	Gizzard shad	18.9	—	2.6	1.1	23
Lake Ontario	Port Ontario, N.Y.	White perch	12.9	—	4.6	1.2	19
Mississippi	Memphis, Tenn.	Drum	11.2	—	4.5	3.4	19
Merrimac	Lowell, Mass.		13.8	75	6.1	3.3	98

one of the forty samples contained less than 1 μg/g PCB residue. The composition of PCB residues in a lake trout and coho salmon were examined by GC-MS and found to be a mixture of Aroclor 1248 and 1254 (Figure 1), and residues of PCBs were in excess of the "DDT" residue levels (Table 2).

Widespread pollution of the major waterways has occurred, and appreciable PCB residues exist in fish. In view of the PCB bioconcentration in fish, PCB levels as high as 5 μg/l may exist in the waters of the Hudson River. These levels are certain to have adverse effects on aquatic organisms.

Toxicity and Bioaccumulation

Fish

Chronic and acute toxicity studies on aquatic organisms are quite limited. Due to the low solubility of PCBs, tests to obtain 96-hr LC_{50} values do not adequately reflect their toxicity to fish (Table 3). For Aroclor 1221-1213, 96-hr LC_{50} values ranged from 1,170 to 20,000 μg/l for cutthroat trout (15). The acute oral toxicity of Aroclors 1242, 1248, 1254, and 1260 was greater than 1500 mg/kg in rainbow trout.

Intermittent-flow bioassays of Aroclor 1242, 1248, and 1254 to bluegills resulted in 15-day

Table 2. Pesticide and PCB Residues in a Coho Salmon and a Lake Trout from Lake Michigan.

	Residues in μg/g whole fish.									
	Dieldrin	p,p' isomers			o,p' isomers		Total "DDT"	PCB (Aroclor)[1]		
		DDE	DDD	DDT	DDE	DDT		1248	1254	Total
Lake trout[2]	0.10	13.0	1.8	5.2	0.9	1.8	22.7	12.0	16.0	28.0
Coho salmon[3]	0.10	4.3	0.8	2.3	0.4	0.5	8.3	6.7	6.7	13.4

[1] Calculated from ratios of characteristic 1248 and 1254 peaks.

[2] Lake trout (33 L)—taken 10-14-69. 690 mm, 3500 gm, immature M. Taken in 2-5 fathoms in Little Bay de Noc, Michigan.

[3] Coho salmon (7Co)—taken 9-3-69. 675 mm, 5718 gm F. Taken in 17-19 fathoms 4 miles NW of Manistee, Michigan.

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LC_{50} values of 51, 76 and 201 μg/l, respectively (table 4). Exposures to channel catfish gave 96-day LC_{50} values of 107, 127, and 741 μg/l for the same Aroclors. All LC_{50} values decreased significantly with longer exposures. The 20-day LC_{50} values for Aroclors 1248, 1254, and 1260 were 10, 135, and 215 μg/l for bluegills and 300, and 200 μg/l for Aroclors 1254 and 1260 with channel catfish at 20°C. The toxicity of Aroclor 1248 to bluegills and channel catfish increased two-fold at 27°C. The toxicity of the PCBs to fish is inversely related to percent chlorine (higher Aroclor number).

In chronic tests to determine the effects of Aroclor 1242 and 1254 on fathead minnow reproduction, all fish exposed to greater than 8.3 μg/l

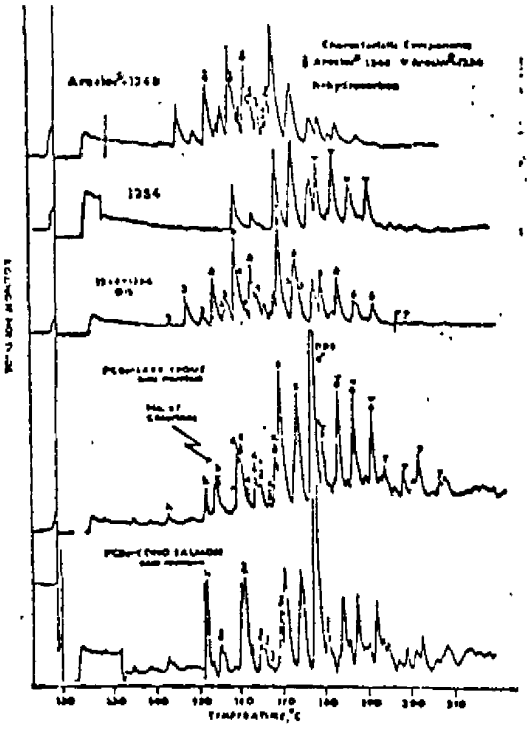

Figure 1. Composition of PCB residues in fish. Arrows and triangles designate the most characteristic peaks of the Aroclor. All numbered peaks in chromatograms of fish extracts are PCBs. The numbers designate the number of chlorine atoms in the compounds as determined by mass spectrometry. Temperature program rate, 4°C/min. He 9 psig, 60"×0.020" i.d. SE-30 SCOT column, Pe-2708 GC-MS.

Table 3. Acute toxicity[1] of several Aroclors to selected fishes.

Aroclor	Species	Temperature (C)	96 hr LC_{50} (μg/l)
1221	Cutthroat trout	8.0[2]	1,170
1232	Cutthroat trout	8.9	2,600
1242	Cutthroat trout	8.0	6,400
1248	Cutthroat trout	8.0	6,700
1254	Cutthroat trout	8.0	42,000
1260	Cutthroat trout	8.9	60,000
1262	Cutthroat trout	8.0	60,000
1268	Cutthroat trout	8.0	60,000
1248	Channel catfish	18.3[3]	6,000
1254	Channel catfish	18.3	12,000
1248	Bluegills	18.3[3]	278
1254	Bluegills	18.3	2,700

[1] Static bioassay.
[2] Alkalinity, 158 ppm; pH 7.6.
[3] Alkalinity, 35 ppm; pH 7.1.

of each Aroclor died. Reproduction occurred at and below 8.3 μg/l Aroclor 1242 and at and below 2.8 μg/l Aroclor 1254 (17).

Accumulation of Aroclor 1248 and 1254 by bluegills chronically exposed to 2-10 μg/l was from 26,300 to 71,400 times the exposure levels for both PCBs. Concentration factors were not strongly dependent upon water exposure levels, but a direct correlation of water concentrations and whole-body residues may exist. No major modification of the PCB isomer ratios was observed in the tissue residues, and no new components were identified (18).

Coho salmon fed Aroclor 1254 for 210 days at concentrations of 14.5 to 14,500 μg/kg body weight per day (dietary concentrations of 0.4 to 580 μg/g) accumulated whole-body residues which were 0.9 to 0.5 times the exposure levels (300 mg/kg highest residue value). Growth rates were not affected, however, all fish exposed to the highest treatment died after 210 days' exposure, and thyroid activity was stimulated in all but the lowest treated group (19).

Direct water exposures appear to represent a greater hazard to fish than dietary exposures, although both contribute to tissue residues. However, in the environment the kinetics of residue uptake from dietary sources could be more important, since PCBs have a high affinity for

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Table 4. Intermittent-flow bioassays of Aroclor against three fishes.[1]

Aroclor	Species	LC_{50} (μg/1) 5 days	10 days	15 days	20 days	25 days	30 days
1254	Rainbow trout	150	8	—	—	—	—
1260		—	240	94	21	—	—
DDT		2.26	0.87	0.26	—	—	—
1242	Bluegills	154	72	54	—	—	—
1248		307	100	70	10	—	—
1254		—	443	201	135	64	—
1260		—	—	—	245	212	151
1242	Channel catfish	—	174	107	—	—	—
1248		—	225	127	—	—	—
1254		—	—	741	300	112	—
1260		—	—	—	206	109	157
1248[2]	Bluegills	137	76	—	—	—	—
1248[2]	Channel catfish	—	94	57	—	—	—

[1] Temperature, 20°C; alkalinity, 200; pH, 7.4.
[2] Temperature, 27°C.

sediments and PCBs can readily enter food chains (20, 21).

Aquatic Invertebrates

Daphnia magna: Aroclor 1248 is the most toxic of the Aroclor series to *Daphnia*, and preliminary studies indicate that levels above 5 μg/1 are not safe for reproduction (17). After 48 hours' exposure to 300 μg/1, *Daphnia* concentrated Aroclor 1254 by 48,000 times (22).

Gammarus pseudolimnaeus: The level of Aroclor 1248 that did not affect reproduction was comparable to that for daphnids, ca 5 μg/1 (17). The 96-hr LC_{50} values for Aroclor 1248 and 1254 were 52 μg/1 and 2,400 μg/1, respectively (Table 5). Scud were more sensitive to Aroclor 1242 (96-hr LC_{50}=10 μg/1). After exposing another species of scud (*Gammarus fasciatus*) to 1.0 μg/1 Aroclor 1254 for 14 days, the PCBs were concentrated 27,000 times the exposure level. No further increase in PCB residue resulted after an additional 21 days of exposure (22).

Orconectes nais: Crayfish were more susceptible to Aroclor 1242 (7-day LC_{50}=30 μg/1) than they were to Aroclor 1254 (7 day LC_{50}=80 μg/1). PCB residues in crayfish did not reach equilibrium after a 28-day exposure to Aroclor 1254, and the uptake was linear during this period (22).

Palaemonetes kadiakensis: Glass shrimp were very sensitive to Aroclor 1254, having a 7-day LC_{50} value of 3 μg/1 compared to a 7-day LC_{50} of 1 μg/1 for DDT (22).

Marine Organisms

Immature pink shrimp were extremely sensitive to Aroclor 1254 exposures. Fifty-one percent of the shrimp died within 15 days when continuously exposed to 0.01 μg/1 Aroclor 1254 (23). Mortalities in two estuarine fishes (*Pogonias rhomboides* and *Leiostomus xanthurus*) exposed for 14 to 45 days to Aroclor 1254 were observed at 5 μg/1. Fish mortalities were not observed at 1 μg/1 during the same exposure time (22). PCB concentration factors in these fish were similar to uptake by freshwater fish ($1–5)\times10^4$ times the exposure levels).

Correlation of Reproduction and PCB Residues

In preliminary investigations, Jensen et al. (23) reported a possible relationship between PCB residues in salmon eggs and egg development in Sweden. On a fat basis, PCB residues in eggs ranged from 7.7 to 34 μg/g and mortality ranged from 16 to 100 percent. A regression analysis of their data resulted in a coefficient of correlation of 0.85, and the correlation was significant at $p < .001$.

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Table 5. 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	160
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	500
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	60

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

[2] Temperature, 15.6°C; alkalinity, 50 ppm; pH, 7.1.

Summary

The occurrence of high levels of PCB residues in aquatic organisms (fish and invertebrates) as complex mixtures of Aroclor formulations has been determined by GC analysis and confirmed by GC-MS. Suitable methods for sample cleanup include gel permeation and silicic acid column chromatography. Agreement on methods for reporting PCB residues is lacking.

The highest concentrations of PCB residues in freshwater fish occur in rivers which are associated with industrialized areas, i.e. Hudson River (ca 200 μg/g whole body residue) and the Ohio and Allegheny Rivers (ca 100 μg/g). Residue levels associated with these rivers approach PCB residues which were associated with fish mortalities in chronic continuous-flow exposures (500-600 μg/g).

Concentration of PCBs by fish is greater than 40,000 times the exposure levels. Similar concentration factors have been observed for invertebrates. Adverse effects on reproduction of aquatic organisms may occur at PCB concentrations of 5 μg/l or less. Further studies are required to determine more definitely the exposure levels which do not adversely effect aquatic organisms.

The problem of PCB residues in industrialized areas can be greatly improved by close monitoring of effluents and by identification of those PCB uses which contribute to effluent contamination. Elimination of widespread PCB residues in fish at concentrations of 1-20 μg/g will require greater efforts, such as the control of PCBs from sewage treatment plants and other as yet unidentified sources of PCBs into the aquatic environment.

Related Materials

Chlorinated naphthalenes are recommended for uses similar to those of the PCBs (27). These materials are recovered by the same analytical procedures as the PCBs (28) and may be recognized by their gas chromatographic elution patterns if PCBs are not present. The materials are designated Halowax 1000, 1031, 1099, 1013, 1014, and 1051 and are listed in an order of increasing chlorine content (27). Aquatic toxicity information is not presently available, nor has

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the persistence and fate of these materials been examined in the aquatic environment.

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