sistance and
uscript.

ication No.

. Am. Med.

rman, S.G.,

e 216, 227

67).

Vos, R.H.,

rlind, G.,

., Bulletin
(1970).

J. Assoc.

foda 22, 9

d Physiol.

(1966).

er, D.L.,
l Meeting,
19 (1970).

Green, J.,

., Fukuoka-

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

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

U. S. Environmental Protection Agency, Gulf Breeze Laboratory
Sabine Island, Gulf Breeze, Florida

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

® Registered trademark, Monsanto Co., St. Louis, Mo.

1 Gulf breeze Contribution No. 120

Bulletin of Environmental Contamination & Toxicology,
Vol. 6, No. 2, 1971, published by Springer-Verlag New York Inc.

DSW 332485

STLCOPCB4079061

METHODS AND PROCEDURES

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

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

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

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

DSW 332486

STLCOPCB4079062

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

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

RESULTS AND DISCUSSION

Mortality

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

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

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

DSW 332487

STLCOPCB4079063

TABLE 1

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

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

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

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

*** milligrams per kilogram

DSW 332488

STLCOPCB4079064

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

Uptake and Retention

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

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

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

CONCLUSIONS

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

*** milligrams per kilogram

DSW 332489

STLCOPCB4079065

TABLE 2

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

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

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

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

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

DSW 332490

STLCOPCB4079066

depending on sample weight).

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

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

ACKNOWLEDGEMENTS

We thank Jerrold Forester and Dennis Knight for conducting residue analyses on many of the samples of fish tissues, and Monsanto Company for supplying the Aroclor 1254.

REFERENCES

1. ANONYMOUS, New Scientist, 32, 612 (1966).

2. D. C. HOLMES, J. H. SIMMONS, and J. O'G. TATTON, Nature, 216, 227 (1967).

3. A. V. HOLDEN and K. MARSDEN, Nature, 216, 227 (1967).

4. R. W. RISEBROUGH, P. REICHE, D. B. PEAKALL, S. G. HERMAN, and M. N. KIRVEN, Nature, 220, 1098 (1968).

5. J. H. KOEMAN, M. C. TEN NOEVER DE BRAUW, and R. H. DEVOS, Nature, 221, 1126 (1969).

6. T. W. DUKE, J. I. LOWE, and A. J. WILSON, JR., Bull. Environ. Contam. and Toxicol. 5, 171 (1970).

7. J. I. LOWE, Trans. Am. Fish. Soc. 93, 396 (1964).

8. P. A. MILLS, J. F. ONLEY, and R. A. GAITHER, J. Ass. Offic. Agr. Chem. 46, 182 (1963).

9. H. F. ENOS (1967 Personal Communication) Perrine Primate Research Laboratory, F. D. A., P. O. Box 490, Perrine, Florida 33157.

10. C. HENDERSON, Q. H. PICKERING, and C. M. TARZWELL, Trans. Am. Fish. Soc. 88, 23 (1959).

11. D. J. HANSEN and A. J. WILSON, JR., Pestic. Monit. J. 4, 51 (1970).

DSW 332491

STLCOPCB4079067