22

MAY 28 1974

Effect of Polychlorinated Biphenyl Compounds on Survival and Reproduction of the Fathead Minnow and Flagfish.

Alan V. Nebeker,[1] Frank A. Puglisi, and David L. DeFoe

U. S. Environmental Protection Agency

National Water Quality Laboratory

6201 Congdon Boulevard

Duluth, Minnesota 55804

Trans. Amer. Fish. Soc. 103(3): July, 1974

---

[1] Present Address: Western Fish Toxicology Station, NWQL, 1350 SE Goodnight Avenue, Corvallis, Oregon 97330.

DSW 028627

STLCOPCB4012589

Abstract

Two 9-month continuous-flow bioassays and several intermediate length continuous-flow tests were conducted to determine safe levels of Aroclor 1242, 1248, and 1254 for the fathead minnow (Pimephales promelas) and Aroclor 1248 for the flagfish, Jordanella floridae. Calculated 96-hour LC50 values for newly hatched fathead minnows were 7.7 µg/liter for Aroclor 1254 and 15 µg/liter for 1242. Three-month-old fatheads had a 96-hour LC50 of 300 µg/liter for 1242. Reproduction occurred at and below 1.8 µg/liter 1254 and at and below 5.4 µg/liter 1242. Newly hatched young were the most sensitive life stage. Growth of young fatheads was also affected above 2.2 µg/liter 1248, and none survived above 5.1 µg/liter after 30 days. Young flagfish did not survive at 1248 concentrations above 5.1 µg/liter and did not grow well above 2.2 µg/liter.

Tissue residues in fathead minnows ranged from 0.7 µg/g 1248 in control fish to 1036 µg/g 1254 in fish held for 8 months in water containing 4.6 µg/liter 1254.

STLCOPCB4012590

Introduction

Polychlorinated biphenyl compounds (PCB's) are among the many exotic chemicals now finding their way into our waters. They are detected in fish and other aquatic organisms at concentrations much higher than those found in the ambient water. Because analytical methodology for detecting and identifying low levels of PCB's in water and aquatic organisms has only recently become available, little is known about the interactions. The acute toxicities of some of the PCB's produced commercially are presently being demonstrated at the Fish-Pecticide Laboratory, USDI, Columbia, Missouri (Stalling 1970); the Gulf Breeze Laboratory, EPA, Gulf Breeze, Florida (Duke et al. 1970); and the National Water Quality Laboratory, EPA, Duluth, Minnesota (Nebeker et al. 1972). The effects of PCB's on reproduction of Daphnia magna, Gammarus pseudolimnaeus, and the midge Tanytarsus dissimilis have been detailed by Nebeker and Puglisi (In press), but no full life-cycle information is available for fish species.

This study was begun in October 1970 to determine the effects of three PCB's, Aroclor 1242, 1248, and 1254, on the survival, growth, and reproduction of the fathead minnow (Pimephales promelas) and the effects of Aroclor 1248 on the survival and growth of the flagfish, Jordanella floridae. Further studies were conducted to determine the effects of Aroclor 1242 and 1254 on maturation, egg production, egg hatch, young production, and survival of young of the fathead minnow. Tissue residues were also determined to measure uptake of PCB's.

STLCOPCB4012591

Materials and Methods

Physical Testing Methods

Three continuous-flow testing units were used, each consisting of five duplicated test concentrations and a control. Stainless steel and glass aquaria were used for all test chambers. Twelve 10-gallon aquaria were used for adult fish in the Aroclor 1254 testing; twelve 5-gallon aquaria were used for Aroclor 1242 testing; and twenty-four 2 1/2-gallon aquaria were used for testing Jordanella and some young fathead minnows. Young fish from spawning tanks were tested in 5- and 2 1/2-gallon aquaria. Three proportional diluters (Mount and Brungs 1967) provided the necessary continuous-flow conditions. A modification of the diluter system, a gas syringe filled with acetone and PCB, was used with a mechanical injector system to deliver the toxicant to the mixing chamber of the diluter. Agitation was maintained by circulating the mixing box contents through a submersible pump. Additional mixing boxes, with two-way and four-way splits to the duplicate test aquaria, provided further mixing of the PCB solutions. All tanks were randomly distributed on the test tables. Raw Lake Superior water was used for all testing.

The diluters delivered 350 ml (1242), 200 ml (1254), and 150 ml (1248) to each tank every 3 minutes; flow rates were set primarily to maintain adequate dissolved oxygen concentrations. These flow rates ensured a complete exchange of water in the test tanks every 4-5 hours. All tanks were cleaned daily, and excess algal and bacterial growths were scraped away weekly.

STLCOPCB4012592

Spawning substrates for fathead minnows consisted of inverted 3-inch-long logitudinal sections of glass quart beverage bottles. Egg hatchability studies were conducted in the same manner as previous fathead studies by Mount (1968), Brungs (1969), and others.

Temperatures were maintained at 24 ± 1 C. Durotest[2] (Optima FS) and wide spectrum Grow-lux fluorescent tubes provided light. The photoperiod of Evansville, Indiana, was maintained, with the adjustments in day length made every 2 weeks, as recommended by the National Water Quality Laboratory Committee on Aquatic Bioassays (Bioassay Committee MS 1971).

DSW 028631

[2] Mention of commercial products does not constitute endorsement by the U. S. Environmental Protection Agency.

STLCOPCB4012593

Biological Methods

All fish were obtained from stocks at the National Water Quality Laboratory in Duluth, Minn. Ninety-six-hour acute tests with various ages of fish, conducted according to standard methods (American Public Health Association 1965), and chronic tests were carried out to obtain information for future calculation of application factors. Measured PCB concentrations were used for all calculations.

Full life-cycle studies with the fathead minnows (Aroclor 1242 and 1254) and the tests with Jordanella (1248) were started with newly hatched young (< 24 hours old). In the 30-day Jordanella test observations on survival were made daily, and growth was measured at the end of the test. Young fathead minnows were tested in the same diluter system, but in different tanks, at the same time to get comparative information on the two species.

Fathead (full life-cycle) testing was begun with 40 newly hatched young per concentration, 20 fish in each aquarium. They were fed twice daily with newly hatched live brine shrimp, frozen brine shrimp, Daphnia, and dry and frozen commercial fish foods. Growth was measured photographically (McKim and Benoit 1971) at 60 and 90 days and at the end of the test. Prior to sexual maturation five spawning substrates were placed in the aquaria, and the fish were thinned to leave 10 fish per tank. The fish that were removed were used to study the effects of PCB's on adenoxine triphosphatase activity (Cutkomp and Koch In press).

STLCOPCB4012594

Eggs laid on the spawning substrates were removed, counted, and placed in egg cups for hatchability testing. Twenty-five or fifty eggs were placed in cups, and the number hatched was recorded. Some of the newly hatched fish were transferred to smaller aquaria at the same concentrations and held for 30 days to determine survival and growth.

STLCOPCB4012595

Analytical Methods

The ploychlorinated biphenyls tested were Aroclor 1242, 1248, and 1254, manufactured by Monsanto Chemical Company. The stock solutions were prepared by weighing the compound and dissolving it in acetone.

Six liters of test water were collected from each test concentration to be analyzed. Three liters were siphoned into each of two 1-gallon glass bottles to provide duplicate samples. Tefion-lined screw caps were constructed for the bottles. A duplicate of the control and at least one other sample were spiked with equal amounts of the toxicant being used in the system. Ten milliliters of concentrated sulfuric acid and 300 ml of glass-distilled methylene chloride were added to each sample. The caps were then tightened and the bottles placed on a mechanical shaker for 10 minutes. The samples were allowed to settle for at least 10 minutes before the aqueous phase was decanted. The organic phase and remaining water were transferred to a separatory funnel and again allowed to settle. The organic layer was drained through solvent-washed anhydrous sodium sulfate into a Kunderna-Danish evaporator. The solvent was evaporated on a steam bath until the entire sample was contained in the 10 ml evaporator receiver. The remaining solvent was evaporated by blowing a gentle stream of dry nitrogen over it until the first sign of dryness. The receiver was stoppered with a cork wrapped in acetone-washed aluminum foil and stored for gas chromatograph (G.C.) analysis.

STLCOPCB4012596

Analysis was done on a Tracor MT-220 gas chromatograph with a 6-foot 1/4-inch OD glass column packed with 3% OV-1 chromosorb W 80/90 mesh. A Coulson conductivity detector was used in the reductive mode without a catalyst. Sixty ml/min helium carrier gas was used with a 10 ml/min helium purge through the furnace when the column was vented. A rate of 40 ml/min of electrolytic hydrogen was used for the reductive pyrolysis. The temperatures of the inlet and all transfer lines were kept at 260 C, and the column varied from 195 C to 205 C depending on the PCB mixture being measured. The pyrolysis furnace was kept at 820 C.

The residue was dissolved in a volume of n-hexane that would give a concentration such that a 50-microliter injection would give at least 25% scale response on a 1-millivolt chart recorder. The solvent was vented to prevent it from contaminating the furnace.

A single well-defined peak (Figure 1), present in all three of the PCB's tested, was used as an internal standard and was compared to the same peak in the "standard." This "quantitation" was done by alternately injecting standards and samples making sure the standards response (peak height) bracketed the response of the sample injected between them. The responses were plotted on linear graph paper, and the unknown quantity was taken from the plot. The calculated concentrations were corrected for recovery by using the ratio of the difference between the spiked and unspiked duplicate samples to the size of the spike. Mean measured concentrations were used for all calculations of biological data.

STLCOPCB4012597

The extraction efficiencies of this method are presented in a previous report (Nebeker et al. 1972). The recovery values based on the single-peak evaluation show little difference in the Aroclors. This indicates that the extraction of the compound represented by the peak may be only slightly affected by the other compounds present.

The variability of this method on duplicate samples is less than 10%, regardless of concentration of toxicant. The variability of G.C. injection was less than 5% for duplicate injections of the standard or the same residue and up to 10% for injections of different volumes of the same residue.

A comparison of nominal and measured concentrations for all three Aroclors (Nebeker et al. 1972) indicated that, except for a few high values, the diluter systems were working well.

A standard, one/two factor analysis of variance program (ANOVA) written for the CDC 3300 was used to test for experimental differences.

Chemical characteristics of the Lake Superior Test water, measured daily or weekly with Standard Methods (American Public Health Association 1965), were as follows: pH, 7.5-8.0; acidity, 2.4-4.0 mg/liter; alkalinity, 40-43 mg/liter (as $CaCO_3$); calcium hardness, 34-36 mg/liter; and total hardness, 44-46 mg/liter.

Terminal residues of Aroclor 1242 and 1254 in fathead minnows were determined by the Fish-Pesticide Laboratory, Columbia, Missouri, using the methods of Stalling and Johnson (1971). Residues of PCB's in feed used in tests was also determined (Table 9).

STLCOPCB4012598

Results

Fathead Minnow - Aroclor 1242

Concentrations of Aroclor 1242 above 10 µg/liter were lethal to newly hatched fry. Calculated 96-hr LC50 values for Aroclor 1242 were 15 µg/liter for newly hatched young fathead minnows and greater than 234 µg/liter (near 300 µg/liter) for 2-month-old fish. All minnows in the long-term study were dead after 96 hours in 51 µg/liter 1242, and none survived to the end of the test (8 months) at 15 µg/liter. Survival of fry was excellent when reared at the same concentrations their parents lived and spawned at. The final weights and total lengths of the fish did not differ significantly at the end of the 8-month test, however, growth of newly hatched fish was retarded prior to their death at the higher PCB concentrations.

Reproduction occurred at and below 5.4 µg/liter 1242 but spawning results and egg production were highly variable (Table 1) even when numbers of males and females were the same in some duplicate tanks. Egg hatchability was also variable but good hatching occurred at 5.4 µg/liter. Eggs were more resistant than fry at 15 and 51 mg/liter. Eggs produced by the controls but maintained at the higher concentrations hatched with good success, but none of the fry survived.

Terminal residues of Aroclor 1242 in fathead minnows exposed for 8.5 months are given in Table 5 (controls - Table 7).

Fathead Minnow - Aroclor 1248

Fathead minnows died rapidly at 18 µg/liter Aroclor 1248, and none was alive at the end of a 30-day test. Seventy-five percent were alive at 5.1 µg/liter, but their weight was only one-third that

DSW 028637

STLCOPCB4012599

of the controls; their final lengths were not significantly different, however.

The final weight of the fish in 2.2 µg/liter was only half that of the controls; final lengths were the same. The results in the two lowest concentrations (0.54 and 0.18 µg/liter) were not significantly different from the controls.

Fathead Minnow - Aroclor 1254

Aroclor 1254 was more toxic to the fathead minnow than 1242 as they did not survive and reproduce above 1.8 µg/liter 1254.

Calculated 96-hr LC50 values for Aroclor 1254 were 7.7 µg/liter for newly hatched young fathead minnows and greater than 33 µg/liter for 2-month-old fish. All fish in the long-term reproduction study were dead after 96 hours in 15 µg/liter 1254 in both tanks. Survival at lower concentrations was not significantly different from the controls. The mean terminal weights of the fish were not significantly different in the various concentrations, but growth in length was delayed at 4.6 µg/liter. Spawning occurred at 1.8 µg/liter, but was significantly less than that at lower concentrations. Spawning was variable during the test, even between duplicate tanks (Table 2).

Egg hatchability and fry survival were good at and below 1.8 µg/liter (Table 3). Eggs produced by the controls and maintained in higher PCB concentrations (15 µg/liter) hatched readily, but all young were dead within 96 hours. Young fish which were held for 30 days in separate tanks at the same PCB concentrations at which they were spawned survived and grew well.

STLCOPCB4012600

Terminal residues of Aroclor 1254 in fathead minnows exposed for 8 months are given in Table 6 (controls - Table 8).

Jordanella - Aroclor 1248

Fish held in 18 µg/liter Aroclor 1248 lived for about 2 weeks before they started to die. One died every 2-3 days until all were dead at the end of the 40-day test. Thirty-five percent were alive at 5.1 µg/liter at the end of the test; mean weight was only 15% that of the controls (Table 4). Mean length at 5.1 µg/liter was 21.8 mm, compared to 24.6 mm for the controls.

The fish in 18 and 5.1 µg/liter 1248 lost their fins and tail almost completely, as if they had been eroded away. The death of the one remaining fish at 18 µg/liter and three fish in 5.1 µg/liter when the temperature dropped from 25 C to 21 C indicates the precarious condition of the fish even though they were still alive.

Discussion

The results obtained from this study indicate that polychlorinated biphenyls are acutely toxic, and cause chronic toxicity at very low levels. The newly hatched larvae apparently are the most sensitive stage in the life cycle, at least for short-term exposure. The eggs were resistant or impermeable to the PCB mixtures. The inhibition by the PCB mixtures of bacterial-fungal growths on the incubating eggs resulted in a much better egg hatch at higher PCB levels. Juvenile fish, when tested for up to 30 days, were much less susceptible than very young fish. Tissue residues were very high in test fish, with some fish containing more than 200,000 times as much PCB as the water they were tested in.

DSW 028640

STLCOPCB4012602

Acknowledgments

We wish to thank Henry Bell, Wesley Smith, and Allan Batterman for aid during testing, Dr. David L. Stalling for residue analysis of fish tissues, and Dr. Kenneth Biesinger and Kenneth Dyram for helpful suggestions and statistical assistance. We also thank Monsanto Chemical Company for their cooperation and for making Aroclor samples available for testing.

DSW 028641

STLCOPCB4012603

Literature Cited

American Public Health Association. 1965. Standard methods for the examination of water and wastewater. 12th ed. New York 769 p.

Bioassay Committee. MS 1971. Recommended bioassay procedure for fathead minnows Pimephales promelas Rafinesque. National Water Quality Laboratory, Duluth, Minn. 13 p.

Brungs, William A., 1969. Chronic toxicity of zinc to the fathead minnow, Pimephales promelas Rafinesque. Trans. Amer. Fish. Soc., 98: 272-279.

Cutkomp, L. K., and R. B. Koch. The sensitivity of fish ATPases to polychlorinated biphenyls. Nature (in press).

Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor 1254) in the water, sediment, and biota of Escambia Bay, Florida. Bull. Env. Cont. Toxicol. 5: 171-180.

McKim, J. M., and D. A. Benoit. 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (Salvelinus fontinalis). J. Fish. Res. Bd. Canada 28: 655-662.

Mount, D. I. 1968. Chronic toxicity of copper to fathead minnows (Pimephales promelas, Rafinesque). Water Res. 2: 215-223.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21-29.

Nebeker, A. V., and F. A. Puglisi. Effect of polychlorinated biphenyls (PCB's) on survival and reproduction of Daphnia, Gammarus, and the midge Tanytarsus. Trans. Amer. Fish. Soc. (In press).

Nebeker, A. V., F. A. Puglisi, and D. L. DeFoe. 1972. Toxicity of polychlorinated biphenyls to fish and other aquatic life. Final report. U. S. Environmental Protection Agency, National Water Quality Laboratory, Duluth, Minn. (Unpublished report.) 73 p.

DSW 028642

STLCOPCB4012604

Stalling, D.L. 1970. Summary of PCB Research at the Fish-Pesticide Research Laboratory. Report of the Annual meeting of the Great Lakes Fishery Comm., June 16-18.

Stalling, D. L., and J. L. Johnson. 1971. Residues of Aroclor 1254 in Scud. Sp. Rep. on PCB's. Fish-Pesticide Laboratory, Columbia, Missouri.

DSW 028643

STLCOPCB4012605

Table 1. Spawning and egg production of the fathead minnow at various concentrations of Aroclor 1242

Mean measured concentration (µg/liter)	Test tanks	Number of males	Number of females	Number of spawnings	Number of spawnings per female	Total eggs produced	Number of eggs per spawning	Number of eggs per female
51	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
15	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
5.4	A	4	5	25	5.0	1,514	61	303
	B	4	6	0	0	0	0	0
2.9	A	5	4	24	6.0	1,923	80	481
	B	4	5	9	1.8	424	47	85
0.86	A	6	4	5	1.3	138	28	35
	B*	–	–	—	—	—	—	—
0.0 (control)	A	1	8	42	5.2	5,350	127	669
	B	3	6	24	4.0	1,288	54	215

*Accidentally killed.

DSW 028644

STLCOPCB4012606

Table 2. Spawning and egg production of the fathead minnow at various concentrations of Aroclor 1254.

Mean measured concentration (µg/liter)	Test tanks	Number of males	Number of females	Number of spawnings	Number of spawnings per female	Total eggs produced	Number of eggs per spawning	Number of eggs per female
15	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
4.6	A	4	5	0	0	0	0	0
	B	1	5	0	0	0	0	0
1.8	A	3	7	14	2.0	1,473	105	210
	B	4	7	1	0.14	22	22	3
0.52	A	4	6	30	5.0	4,142	138	690
	B	4	6	33	5.5	2,538	77	423
0.23	A	1	8	35	4.4	2,993	86	374
	B	5	5	8	1.6	346	43	69
0.0 (control)	A	3	6	27	4.5	2,838	105	473
	B	4	6	2	0.3	206	103	34

DSW 028645

STLCOPCB4012607

Table 3. Egg hatchability and fry survival of the fathead minnow at various concentrations of Aroclor 1254

Mean measured concentration (µg/liter)	Test tanks	Number of eggs used	Number of live fry obtained	Egg hatchability (%)
15	A	0	0	0
	B	0	0	0
4.6	A	0	0	0
	B	0	0	0
1.8	A	300	186	62
	B	50	48	96
0.52	A	375	251	67
	B	345	205	59
0.23	A	272	150	55
	B	0	0	0
0.0 (control)	A	250	191	76
	B	150	107	71

DSW 028646

STLCOPCB4012608

Table 4. Survival and growth of newly hatched *Jordanella floridae* after 40 days' exposure to Aroclor 1248

Mean measured concentration (µg/liter)	Initial number of animals	Mean survival (%)	Final mean weight (g)	Final mean length (mm)
18	20	0	—	—
5.1	20	35	.60	21.8
2.2	20	85	3.02	24.1
0.54	20	100	4.47	26.3
0.18	20	90	3.90	25.5
0 (control)	20	100	4.33	24.6

STLCOPCB4012609

Table 5. Terminal residues of Aroclor 1242 in fathead minnows exposed for 8.5 months.

Mean measured PCB concentration (µg/liter)	Residues (µg/g)						Concentration factor	
	A		B		Mean			
	Males	Females	Males	Females	Males	Females	Males	Females
0.86	62(2)	92(3)[a]	—	—	236	92[c]	274,000	107,000
	410[4][b]	—	—	—				
2.9	—	—	124(3)	98(3)	93	107	32,000	37,000
	—	—	62[5]	117[5]				
5.4	306(1)	364(2)	—	436(2)	345	436	63,000	81,000
	278[2]	430[4]	452[2]	514[5]				

[a] Mean of (N) individual samples.
[b] Pooled samples of [N] fish.
[c] Average of pooled and mean individual analysis for both A and B tests.

DSW 028648

STLCOPCB4012610

Table 6. Terminal residues of Aroclor 1254 in fathead minnows exposed for 8 months.

Mean measured PCB concentration (µg/liter)	Residues (µg/g) A Males	A Females	B Males	B Females	Mean Males	Mean Females	Concentration factor Males	Concentration factor Females
0.23	57(2)	28(1)	43(2)[a]	—	54	45[c]	235,000	196,000
	—	48[6]	61[4][b]	60[4]				
0.52	133(2)	99(3)	64(2)	104(2)	81	105	156,000	201,000
	54[3]	--	74[4]	113[5]				
1.8	204(1)	458(3)	103(1)	361(2)	196	429	109,000	238,000
	83[2]	347[6]	394[4]	553[5]				
4.6	803(1)	741(1)	1110(1)	1004(5)	1036	999	225,000	217,000
	1204[3]	1253[3]	—	—				

[a] Mean of (N) individual samples.
[b] Pooled samples of [N] fish.
[c] Average of pooled and mean individual analysis for both A and B tests.

DSW 028649

STLCOPCB4012611

Table 7. Terminal residues in control fish from Aroclor 1242 study

Fish (µg/g)	P,P'-DDE	Aroclor 1248	Aroclor 1260
A controls			
Males (1)[a]	0.02	--	2.2
Females (8)	0.08	3.3	2.8
B controls			
Males (3)	0.04	0.9	1.1
Females (6)	0.10	3.2	2.8

[a]Average of (N) fish.

STLCOPCB4012612

Table 8. Terminal residues in control fish from Aroclor 1254 study

Fish (ug/g)	p',p'-DDE	Aroclor 1248	Aroclor 1260
A controls			
Males (3)[a]	0.05	0.7	1.1
Females (6)	0.04	1.1	2.3
B controls			
Males (2)	0.03	0.9	1.9
Females (8)	0.04	1.6	2.7

[a]Average of (N) fish.

STLCOPCB4012613

Table 9. Residues in feed used in tests

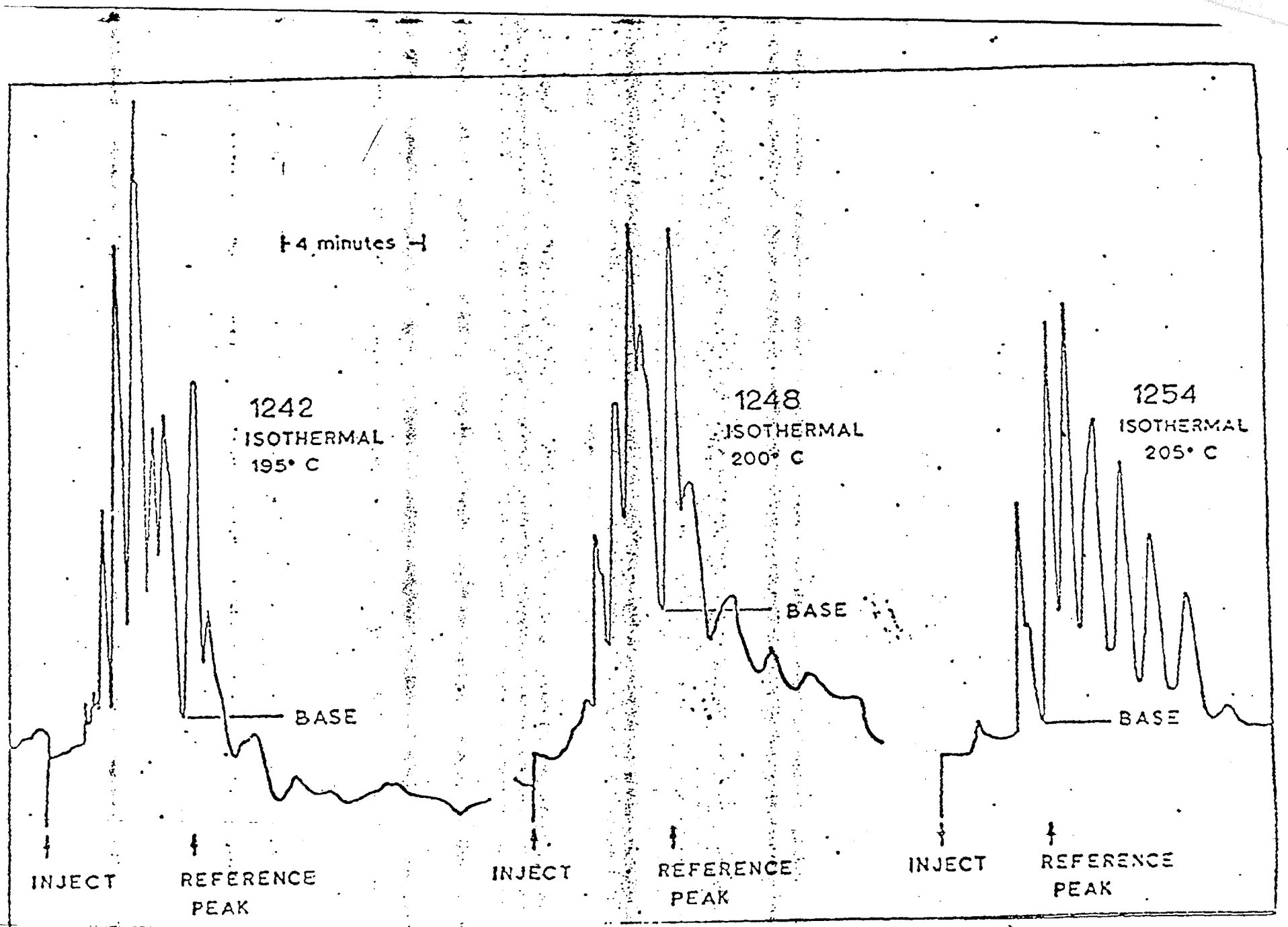
	Pesticide and PCB (µg/g)				
	p,p'-DDE	p,p'-DDD	p,p'-DDT	Aroclor 1248	Aroclor 1254
Fish food Jan., 1971	0.22	0.02	0.10	0.3	0.8
Fish food Feb., 1971	0.20	0.02	0.10	0.5	1.5

DSW 028652

STLCOPCB4012614

Figure 3. Single peak used as reference for quantitation of Aroclors 1242, 1248, 1254.

STLCOPCB4012615



Nebeker, Purdiat and DeFoe

STLCOPCB4012616