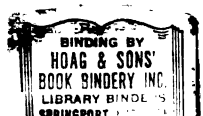
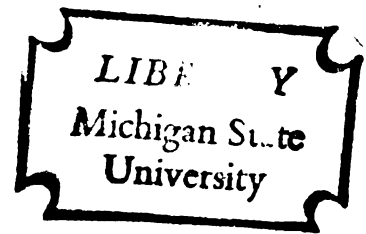


THE ACUTE TOXICITY OF
A POLYCHLORINATED BIPHENYL,
AROCOR 1254, TO THE EARLY
LIFE STAGES OF COHO SALMON
AND STEELHEAD TROUT

Thesis for the Degree of M. S.
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ABSTRACT

THE ACUTE TOXICITY OF A POLYCHLORINATED BIPHENYL, AROCLOR 1254, TO THE EARLY LIFE STAGES OF COHO SALMON AND STEELHEAD TROUT

By

Mark Thomas Halter

Some acute effects of Aroclor 1254 (PCB) to the early life stages of coho salmon (Onchorhynchus kisutch (Walbaum)) and steelhead trout (Salmo gairdneri irideus Gibbons) were investigated using continuous flow bioassay techniques.

PCB did not influence the acute toxicity of DDT to 5-10 week old coho salmon of Michigan or Oregon parent stock. In 14 day bioassays, fish were exposed to 45.0 to 3.0 $\mu\text{g/l}$ PCB or 3.3 to 0.2 $\mu\text{g/l}$ DDT and combinations of 45.0 + 3.3 to 3.0 + 0.2 $\mu\text{g/l}$ PCB + DDT. PCB-DDT combinations produced no greater mortalities than could be expected from exposure to the DDT concentrations alone. The rapidity of DDT toxicity with respect to that of PCB was suggested as the reason for lack of interaction. Michigan salmon were more resistant to DDT than Oregon salmon. Symptoms of PCB and DDT poisoning were described.

Coho salmon exposed to 56.40 to 4.35 $\mu\text{g/l}$ Aroclor 1254 from the last two weeks of egg stage until four weeks beyond

hatching were adversely affected at all exposure levels.

Salmon exposed to the same PCB levels during the last two weeks of the egg stage only were adversely affected at levels above 15 $\mu\text{g}/\text{l}$. Egg hatchability, mean incubation time, fry survival, and fry growth were measured. All egg groups exposed to PCB hatched prematurely.

Two month old steelhead trout accumulated PCB at 0.189, 1.044, 1.832, and 3.106 $\mu\text{g}/\text{g}$ per hour when exposed to 3.25, 10.45, 27.80, and 51.30 $\mu\text{g}/\text{l}$ Aroclor 1254 for 24 days. Uptake rates could be predicted given one uptake rate from a known exposure level. The fish concentrated PCB 32,000 to 38,000 times over the exposure concentration but plateaus were not reached. Bioconcentration factors in fish at 100 hour time intervals through the test were found to be inversely related to the exposure concentration. Fish brains accumulated PCB at a slower rate than the whole body beyond 200 hours exposure or after accumulating about 400 $\mu\text{g}/\text{g}$ PCB.

In a thirty day toxicity test the median survival times of 100 day old steelhead trout exposed to 39.40 and 20.40 $\mu\text{g}/\text{l}$ Aroclor 1254 were 293 and 400 hours, respectively. The growth of fish exposed to more than 4.70 $\mu\text{g}/\text{l}$ PCB was reduced.

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TABLE OF CONTENTS

	Page
INTRODUCTION.	1
GENERAL METHODS AND MATERIALS	5
PCB-DDT COMBINATION BIOASSAYS	13
Methods and Materials.	13
Results.	18
Discussion	33
COHO SALMON EGG-ALEVIN EXPOSURE	35
Methods and Materials.	35
Results.	36
Discussion	39
PCB UPTAKE BY STEELHEAD TROUT	41
Methods and Materials.	41
Results.	43
Discussion	53
STEELHEAD TROUT TOXICITY BIOASSAY	57
Methods and Materials.	57
Results.	59
Discussion	59
CONCLUSION.	64
LITERATURE CITED.	67
APPENDIX--BREAKDOWN OF LOSSES OF EGG, SAC-FRY AND ALEVINS EXPOSED TO VARIOUS CONCENTRATIONS OF AROCLOR 1254	72

LIST OF TABLES

TABLE	Page
1. Chemical characteristics of the water used in all bioassays.	9
2. The toxicant concentrations, in $\mu\text{g}/\text{l}$, present in the test tanks of the PCB-DDT bioassays performed with Michigan coho salmon	16
3. The toxicant concentrations, in $\mu\text{g}/\text{l}$, present in the test tanks of the PCB-DDT bioassays performed with Oregon coho salmon	17
4. Summary of toxicity data for Michigan coho salmon exposed to various concentrations of PCB, DDT or PCB-DDT combinations.	19
5. Summary of toxicity data for Oregon coho salmon exposed to various concentrations of PCB, DDT or PCB-DDT combinations	20
6. Summary of test in which eyed coho salmon eggs were incubated, hatched, and the resulting alevins allowed to develop for four weeks in various concentrations of Aroclor 1254	37
7. Summary of test in which eyed coho salmon eggs were incubated for two weeks in various concentrations of Aroclor 1254, then transferred to an uncontaminated system for hatching and development.	38
8. The Aroclor 1254 concentrations, in $\mu\text{g}/\text{l}$, present in the test tanks during the PCB uptake study as determined by gas chromatography.	42
9. Actual magnification factors calculated by substitution of the appropriate number of hours into the regression equations describing PCB uptake	52

LIST OF TABLES--Continued

TABLE	Page
10. Magnification factors calculated by substitution of the appropriate number of hours into the regression equations, excluding the "Y" intercept value, describing PCB uptake.	52
11. The Aroclor 1254 concentrations, in $\mu\text{g}/\text{l}$, present in the test tanks during the thirty day toxicity test as determined by gas chromatography.	58
12. The length and weight of steelhead trout before and after exposure to various concentrations of Aroclor 1254	62

LIST OF FIGURES

FIGURE	Page
1. The toxicant introduction system of the flow-through test apparatus used in the present studies.	7
2. Gas chromatogram of Aroclor 1254	12
3. The toxicity of various concentrations of PCB and PCB-DDT combinations to young Michigan coho salmon.	22
4. The toxicity of various concentrations of DDT and PCB-DDT combinations to young Michigan coho salmon.	24
5. The toxicity of various concentrations of DDT and PCB-DDT combinations to young Oregon coho salmon	26
6. The toxicity of various concentrations of DDT and PCB-DDT combinations to young Oregon coho salmon	28
7. The toxicity of various concentrations of PCB and PCB-DDT combinations to young Oregon coho salmon	30
8. A comparison of standard and tissue samples chromatograms of Aroclor 1254.	45
9. The uptake of Aroclor 1254 from water by young steelhead trout.	47
10. Aroclor 1254 concentrations in the bodies and brains of young steelhead trout killed by exposure to 51.30 µg/l Aroclor 1254.	50
11. The results of a thirty day toxicity test in which young steelhead trout were exposed to five concentrations of Aroclor 1254.	61

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INTRODUCTION

Monitoring studies have revealed that residues of two chlorinated hydrocarbon compounds, DDT and polychlorinated biphenyls (PCB), are prevalent in the Lake Michigan ecosystem (Lake Michigan Interstate Pesticide Committee, 1972; Johnson and Ball, 1972; Reinert, 1970; Hickey et al., 1966). Concentrations of DDT and PCB in off-shore waters of the lake are commonly estimated at 1-10 and 1-20 ng/l (pptr), respectively, while inshore and tributary waters near agricultural, urban, or industrial centers may be somewhat more contaminated (Inter-Departmental Task Force on PCBs, 1972; Lake Michigan Interstate Pesticide Committee, 1972). The bulk of residues entering the lake are thought to be sorbed onto bottom sediments (Nisbet and Sarofim, 1972) and may be available for continuous equilibria exchange with the ambient water (Hamelink et al., 1971) or slow microbial degradation (Matsumura et al., 1971).

Various fish species in Lake Michigan have whole body DDT residues of 2-20 $\mu\text{g/g}$ and PCB levels are often about twice this amount (Lake Michigan Interstate Pesticide Committee, 1972; Reinert, 1970; Henderson et al., 1970). Residues in salmon and trout are frequently near the upper limits of these

ranges, apparently due to their high fat content and top position in the food chain (Reinert, 1970). DDT and PCB residues in excess of 5 $\mu\text{g/g}$ have been the basis for actions or warnings by the Food and Drug Administration in 1968 and 1971 against Lake Michigan commercial fishery products and food fish taken by sports fishermen. The Lake Michigan Interstate Pesticide Committee (1972) estimated that under presently enforced FDA guidelines about 80% of the total annual catch from the lake (which in 1967 amounted to 59 million pounds (Lyles, 1967) is nonmarketable in interstate commerce.

The reproductive potential of coho salmon in Lake Michigan may be impaired by chlorinated hydrocarbons transferred from adult fish to their eggs. Johnson and Pecor (1969) described a mortality syndrome associated with significant losses among coho salmon sac-fry reared in Michigan hatcheries. The losses typically occurred during the seventh week after hatching at the time of final yolk sac absorption. These authors correlated the appearance of symptoms in affected fry with DDT residues in the body and gut tissues. However, the correlations have not always been consistent among different fry groups or from year to year. Johnson (1972) suggested that PCB residues present in the fry may influence overall survival.

Toxic interactions between DDT and PCB were demonstrated with houseflies by Lichtenstein et al., (1969) who found that

topical applications of 500 µg/g of several PCB formulations in combination with 15 µg/g of DDT produced markedly higher mortalities than either compound applied alone. These results have also been repeated with pesticides other than DDT (Fuhremann and Lichtenstein, 1972). Lichtenstein (1969, 1972) warned that biological interactions between PCBs and other synthetic chemicals present in biological systems are possible and should not be disregarded.

Although PCBs have been manufactured since 1929, their presence in Lake Michigan and in other ecosystems was not anticipated or forewarned as was the case with DDT. Consequently, they remained undiscovered until 1966. Therefore, information regarding the effects of PCB on aquatic life was unavailable until only recently (Interdepartment Task Force on PCBs, 1972; National Institute of Environmental Health Sciences et al., 1972).

The present study was conducted to provide basic toxicological information about the acute effects of PCB on the early life stages of coho salmon (Onchorhynchus kisutch (Walbaum)) and steelhead trout (Salmo gairdneri irideus Gibbons). Specifically, the test objectives were: 1) to compare the acute toxicities of PCB, DDT and PCB-DDT combinations to young coho salmon; 2) to determine the effect of PCB exposure on coho eggs and alevins; 3) to determine the rate of uptake of PCB by young trout; and 4) to determine levels of PCB toxic to young trout over a thirty day period.

A single PCB formulation, Aroclor 1254 (Monsanto Company, St. Louis, Missouri), was used exclusively in these experiments and will be referred to as PCB in this thesis. Aroclor 1254 is the trade name for a mixture of biphenyl rings averaging 54% chlorine substituents by weight. This PCB was selected for testing because its gas chromatographic tracing most closely resembles that of the residues extracted from water and fish of Lake Michigan (Veith, 1972; Armour and Burke, 1970).

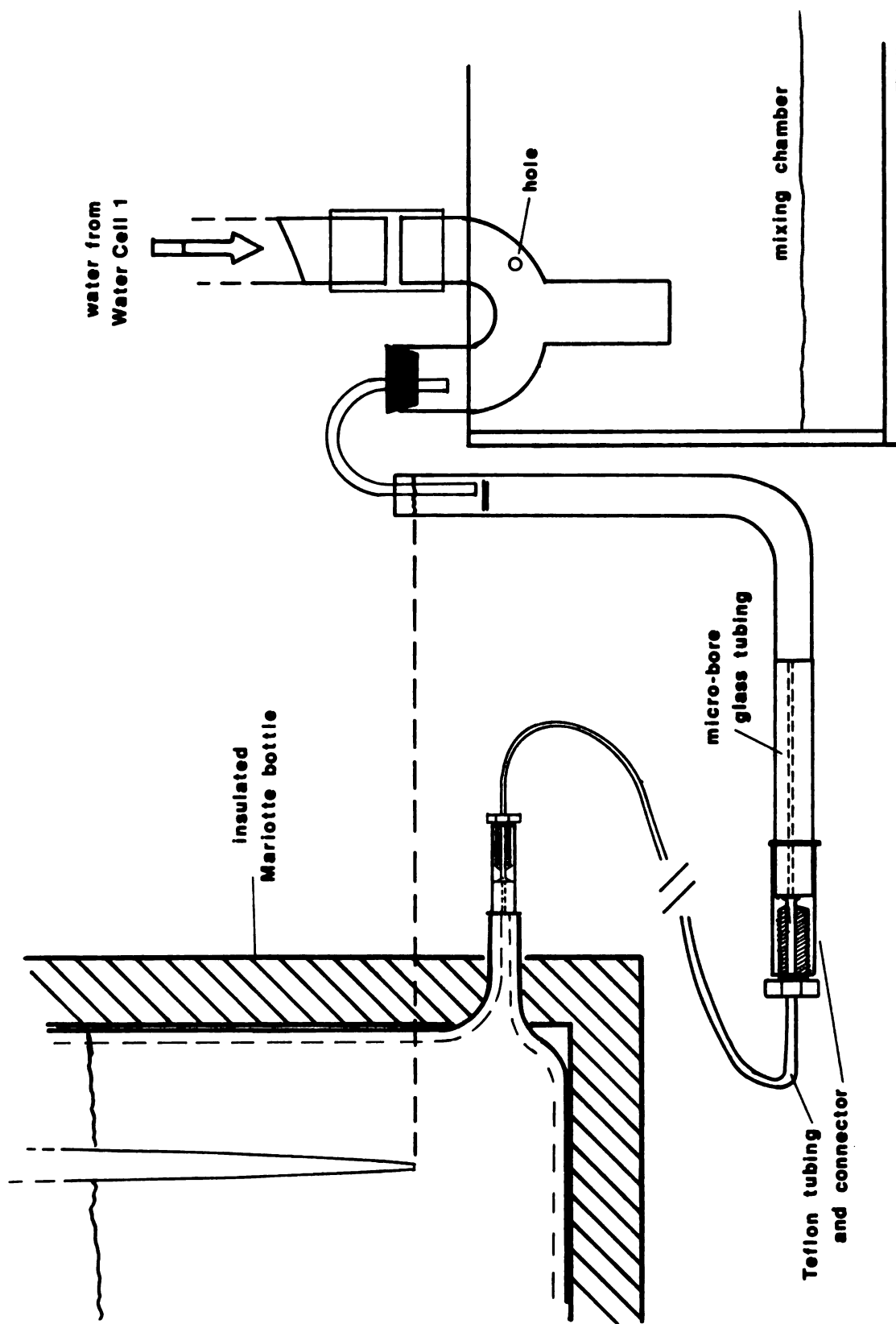
GENERAL METHODS AND MATERIALS

Steelhead trout and coho salmon were reared in the laboratory from eggs collected in April and October of 1971 respectively, at weir sites operated by the Michigan Department of Natural Resources on the Platte river, a tributary of Lake Michigan located in Benzie County, Michigan. Eyed coho salmon eggs were also received in November, 1971, via air shipment, from the Oregon Fish Commission. In each lot, the eggs of several females were fertilized with the sperm of more than one male.

Young fish were held in tanks supplied by the same water source as used in all tests. Michigan fish were maintained on Ewos starter diet, a Swedish pelleted dry food. Oregon salmon were fed Oregon Moist diet.

All exposures were conducted using proportional diluters (Mount and Brungs, 1967) delivering five toxicant concentrations plus a control at a rate of 200 ml/min. Modified versions (Figure 1) of the toxicant introduction system of McAllister et al. (1972) were calibrated to deliver either 0.25 or 1.0 ml of stock solution to the mixing chambers of the diluters with each diluter cycle. The delivery tubes from the diluters were randomly distributed to convenient

Figure 1. The toxicant introduction system of the flow-through test apparatus used in the present studies. The basic design is adapted from McAllister et al. (1972).



TOXICANT DELIVERY SYSTEM

Figure 1

arrangements of screen-covered glass aquaria which had stand-pipe controlled water volumes of 14 liters. Water replacement time (90%) in the test tanks was approximately $2\frac{1}{2}$ hours. The rate of water flow per gram of fish in these bioassays was never less than 4 l/g per day.

Toxicant stock solutions were made up in four liters of reagent grade acetone with appropriate amounts of Aroclor 1254 and transferred directly to the Mariotte bottle of the toxicant introduction system. After dilution the nominal test concentrations of Aroclor 1254 in all tests were 80, 40, 20, 10, and 5 $\mu\text{g/l}$. Nominal acetone concentrations present when 1.0 ml of stock solution was delivered with each diluter cycle were 800, 400, 200, 100, and 50 mg/l; or 200, 100, 50, 25, and 12.5 mg/l if 0.25 ml of stock was delivered.

Dechlorinated Michigan State University tap water (Table 1) was used in all tests. Routine measurements of hardness, total alkalinity, and pH over the one year test period revealed no significant fluctuation in these characteristics. Dissolved oxygen, measured in the test tanks during each exposure, ranged from 7.3 to 8.5 mg/l. Water temperature was maintained at 12-14°C by a Min-O-Cool aerator in a 100 gallon reservoir through which the test water passed prior to use. The laboratory was continuously lighted by overhead fluorescent lamps.

Actual test concentrations of Aroclor 1254 were determined by gas-liquid chromatography (GLC) using a Micro-Tek

Table 1. Chemical characteristics of the water used in all bioassays.

Parameter	Conc. (mg/l)
Hardness as CaCO_3	336
Total Alkalinity as CaCO_3	318
Ammonia Nitrogen	0.24
Chloride	5.8
Cu, Fe, Pb, Zn	<0.01
Nitrate	0.01
Phosphorus	0.87
Sulfate	3.0
pH	7.3
Conductivity as $\mu\text{mho}/\text{cm}^2$	682

220 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a $\frac{1}{4}$ inch by 6 foot glass column packed with 3 percent SE-30 on 60-80 mesh Gas Chrom-Q. Inlet, column, and detector temperatures were 205, 175, and 365°C, respectively and the nitrogen carrier gas flow was 85 ml/min. One liter water samples collected from individual test chambers were prepared for analysis by successive extractions with 100, 50 and 50 ml of redistilled hexane. The combined extract was then dried over anhydrous sodium sulfate, reduced to volume and a known amount injected into the gas chromatograph. Standard solutions were injected after every one to three samples.

Quantitation of Aroclor 1254 was based on the combined heights of peaks 4 through 9 (shown in Figure 2). A comparison of water extract chromatograms to those of standards indicated these specific peaks were stable and unmodified in water solution.

Recoveries from eight one liter water samples spiked with 25 to 50 $\mu\text{g/l}$ of Aroclor 1254 averaged 83%. The measured test concentrations given in this paper have not been corrected for this extraction efficiency. The remainder of the difference between nominal and measured concentration values reported is primarily due to the sorption of PCB onto the various surfaces of the test apparatus which the test solutions contacted.

Figure 2. Gas chromatogram of Aroclor 1254. The combined heights of peaks 4 through 9 were used for quantitation of PCB concentrations in water and fish tissue samples.

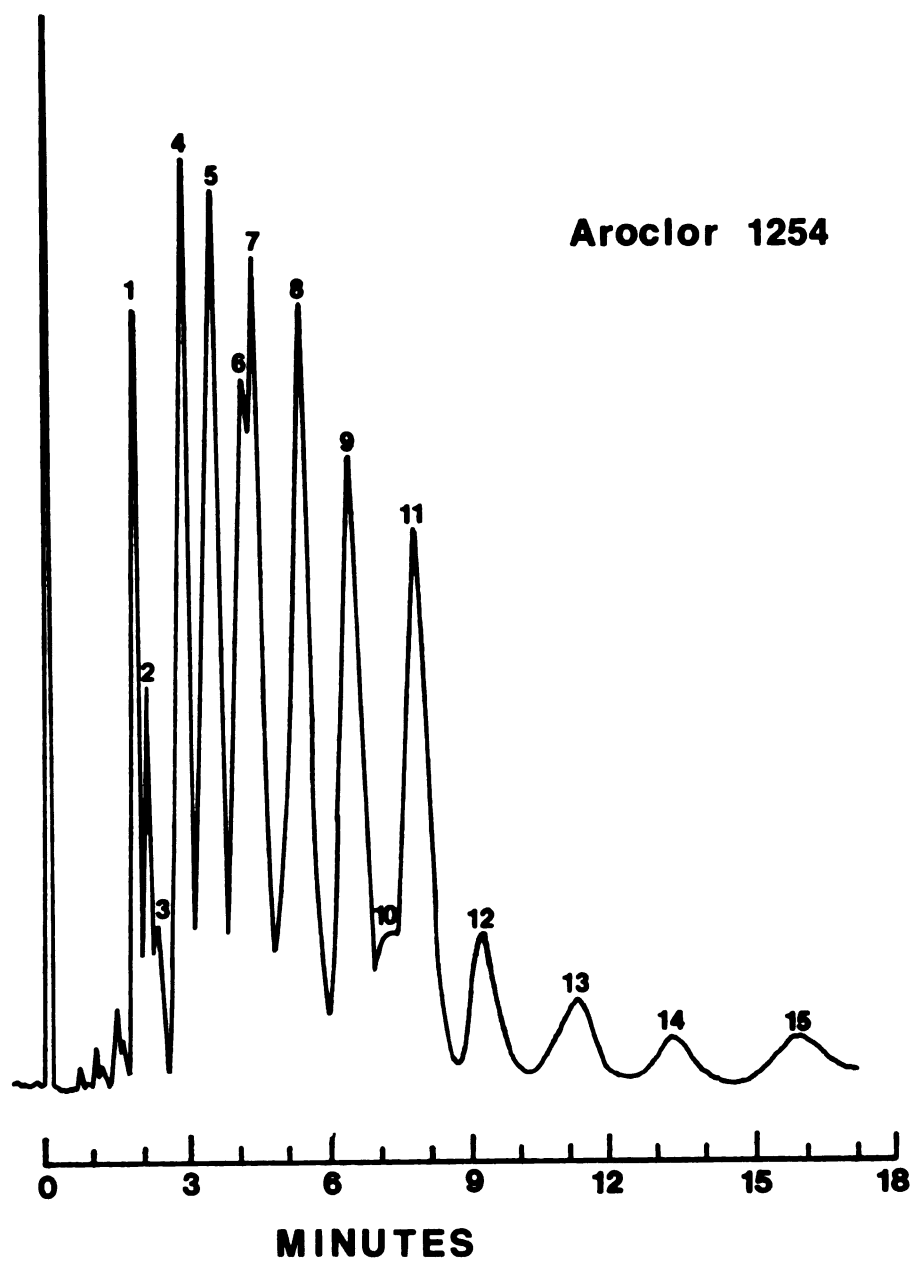


Figure 2

PCB-DDT COMBINATION BIOASSAYS

Methods and Materials

Four pairs of 14 day bioassays, and one pair of 7 day bioassays were conducted using two diluters equipped with 0.25 ml toxicant introduction devices. One system delivered PCB-DDT mixtures while the other delivered concentrations of PCB or DDT alone. A "dipping bird" apparatus (Mount and Brungs, 1967) was used to maintain 200 mg/l acetone in the control tank of each system. The first two bioassays tested Michigan salmon and the last three tested Oregon salmon.

DDT (100% p,p' 1,1,1, trichloro 2,2 bis (-p chloro-phenyl) ethane) stock solutions were made up in the same manner described in the general methods for PCB stock solutions. The nominal DDT test concentrations in all bioassays were 5, 2.5, 1.25, 0.63, and 0.31 µg/l. When combinations were tested, PCB and DDT were mixed in the same stock bottle to give nominal test concentrations of 80+5, 40+2.5, and so on, µg/l PCB-DDT.

In each test the diluters were calibrated and run normally for 12-15 hours (overnight). Twenty-five fry were then randomly assigned to each of the twelve test chambers. Each day for two weeks the fish were observed at least four times

between 9 a.m. and 10 p.m. Dead fish were removed as observed and the times of mortality were recorded. The fish were fed a measured amount of food three times daily. The food ration was reduced but not discontinued for fish groups no longer accepting food. Uneaten food and feces were siphoned from each tank daily. At the end of the test, surviving fish were sacrificed, measured and weighed.

Between tests the diluters, delivery tubes, and aquaria were washed with hot water and detergent and rinsed twice with acetone.

Starting January 25, 1972, Michigan salmon about 35 days old were treated with PCB alone and PCB-DDT combinations and this test was followed with a bioassay of DDT alone and the PCB-DDT combinations. Oregon salmon bioassays, begun on February 27, first tested DDT alone and the PCB-DDT combinations. This two week test was then repeated with a one week exposure. Finally, Oregon salmon were tested with PCB alone and the PCB-DDT combinations.

During the Michigan salmon bioassays, water samples for toxicant determination were taken from half of the test tanks of each exposure system at the beginning, middle, and end of each test. This sampling scheme was increased to sampling all tanks every three days during the Oregon salmon bioassays to more adequately define actual toxicant exposure concentrations.

The water samples were analyzed (Tables 2 and 3) by gas-liquid chromatography as described in the general methods section. Quantitation of PCB alone was based on the heights of peaks 4 through 9 (Figure 2) while that of DDT was based on its single peak height. In water samples of PCB-DDT combinations, both compounds were quantified without separation. Under the described gas chromatographic conditions, the retention time of DDT was identical to that of the isomer(s) causing peak 11 on the chromatogram of Aroclor 1254. The normal height of peak 11 relative to that of adjacent peak 9 was known from experience with many chromatograms of Aroclor 1254 extracted from water. Therefore when the enlarged peak 11 of a PCB-DDT chromatogram was measured, peak 9 on the same chromatogram was used to estimate the portion of peak 11 due to PCB and the remainder was taken as the peak height of the DDT in the sample. The latter was then used for quantitation of DDT by comparison to a standard plot of DDT peak height vs. nanograms injected, obtained by injecting known amounts of DDT and PCB mixtures into the gas chromatograph. PCB concentrations in the combination samples could be determined in the usual manner since DDT did not interfere with peaks 4 through 9.

The average recoveries from twenty-three samples of water spiked with 5 to 50 $\mu\text{g/l}$ of PCB and/or DDT were 82-84% for each compound.

Table 2. The toxicant concentrations, in µg/l, present in the test tanks of the PCB-DDT bioassays performed with Michigan coho salmon.

NOMINAL CONCENTRATIONS									
PCB or DDT:		80.00 or 5.00	40.00 or 2.50	20.00 or 1.25	10.00 or 0.63	5.00 or 0.31			
PCB + DDT:		80.00 + 5.00	40.00 + 2.50	20.00 + 1.25	10.00 + 0.63	5.00 + 0.31			
MEASURED CONCENTRATIONS									
Test 1									
PCB:		47.60	28.25	11.35	-----	4.06			
PCB + DDT:		41.00 + 2.75	20.00 + 1.50 ^a	10.30 + 0.65	5.95 + 0.35	3.55 + 0.39			
Test 2									
DDT:		3.10	2.15	0.95	0.40	0.25			
PCB + DDT:		44.45 + 2.80	24.00 + 1.50	9.85 + 0.55	4.80 + 0.40	2.64 + 0.20			

^aApproximate toxicant concentrations. Actual concentrations not determined.

Table 3. The toxicant concentrations, in $\mu\text{g/l}$, present in the test tanks of the PCB-DDT bioassays performed with Oregon coho salmon.

NOMINAL CONCENTRATIONS						
PCB or DDT:	80.00 or 5.00	40.00 or 2.50	20.00 or 1.25	10.00 or 0.63	5.00 or 0.31	
PCB + DDT:	80.00 + 5.00	40.00 + 2.50	20.00 + 1.25	10.00 + 0.63	5.00 + 0.31	
MEASURED CONCENTRATIONS						
Test 3						
DDT (+SD):	3.20+0.68	1.40+0.16	0.80+0.12	0.45+0.18	0.25+0.20	
PCB + DDT:	40.90+2.70	20.75+1.25	11.70+0.80	6.70+0.45	3.70+0.30	
(+SD +SD)	(+3.86 +0.32)	(+4.19 +0.32)	(+0.87 +0.11)	(+1.45 +0.08)	(+1.89 +0.04)	
Test 3.5						
DDT (+SD):	3.30+1.00	1.90+0.05	0.70+0.03	0.50+nc	0.25+nc	
PCB + DDT:	44.60+2.15	24.15+1.15	12.80+0.60	6.95+0.40	3.80+0.20	
(+SD +SD)	(+10.21+0.54)	(+9.97+0.25)	(+0.84+0.04)	(+0.10+nc)	(+0.85+nc)	
Test 4						
PCB (+SD):	32.20+7.80	16.50+4.11	8.85+2.27	4.10+0.80	2.10+0.30	
PCB + DDT:	33.00+2.00	15.25+0.80	9.85+0.45	4.30+0.25	2.85+0.16	
(+SD +SD)	(+3.18+0.77)	(+3.48+0.15)	(+1.66+0.06)	(+0.65+0.05)	(+0.85+0.04)	

nc = not calculated

Mean toxicant concentrations from individual test tanks within each test system were compared for significant differences by the Student t-test at $p = 0.05$. Median survival times (MST) with 95% confidence limits were calculated for fish in each concentration in each bioassay by the method of Litchfield (1949).

Results

DDT was more toxic than Aroclor 1254 to the young coho salmon (Tables 4 and 5, Figures 3 to 7). Median survival times of fish exposed to more than 1 $\mu\text{g}/\text{l}$ DDT were always less than 336 hours while Aroclor 1254 concentrations below about 30 $\mu\text{g}/\text{l}$ failed to kill half of the test fish within 336 hours. Median survival times of fish groups exposed to PCB-DDT combinations was always less than 336 hours if the DDT level was greater than 1 $\mu\text{g}/\text{l}$. In no case, however, was the median survival time of the fish in PCB-DDT combinations less than that of fish in the next highest DDT concentration within the same test.

Comparison of the tests in which Michigan and Oregon salmon of similar body weight were exposed to similar toxicant concentrations show that the Michigan fish had longer median survival times.

During the final stages of yolk-sac absorption, a general mortality occurred among all Michigan coho salmon, producing a 40% loss. The symptoms were similar to those described by

Table 4. Summary of toxicity data for Michigan coho salmon exposed to various concentrations of PCB, DDT or PCB-DDT combinations.

Test No.	Fish Data		Test Conc.		Median Survival Times (MST) (hours)	95% Confidence Interval (hours)
	Start	Finish	(µg/l)	DDT		
1			41.00+2.75		88	77-101
	\bar{X} length (mm)	36 40	20 +1.50 ^a		120	101-142
	\bar{X} weight (gm)	0.33 0.50	10.20+0.65		200	178-223
			47.60 --		196	160-240
			28.25 --		> 336	----
2			-- 3.10		34	31-38
	\bar{X} length (mm)	40 48	44.50+2.80		80	65-98
	\bar{X} weight (gm)	0.50 1.00	24.00+1.50		115	88-149
			-- 0.95		> 336	----

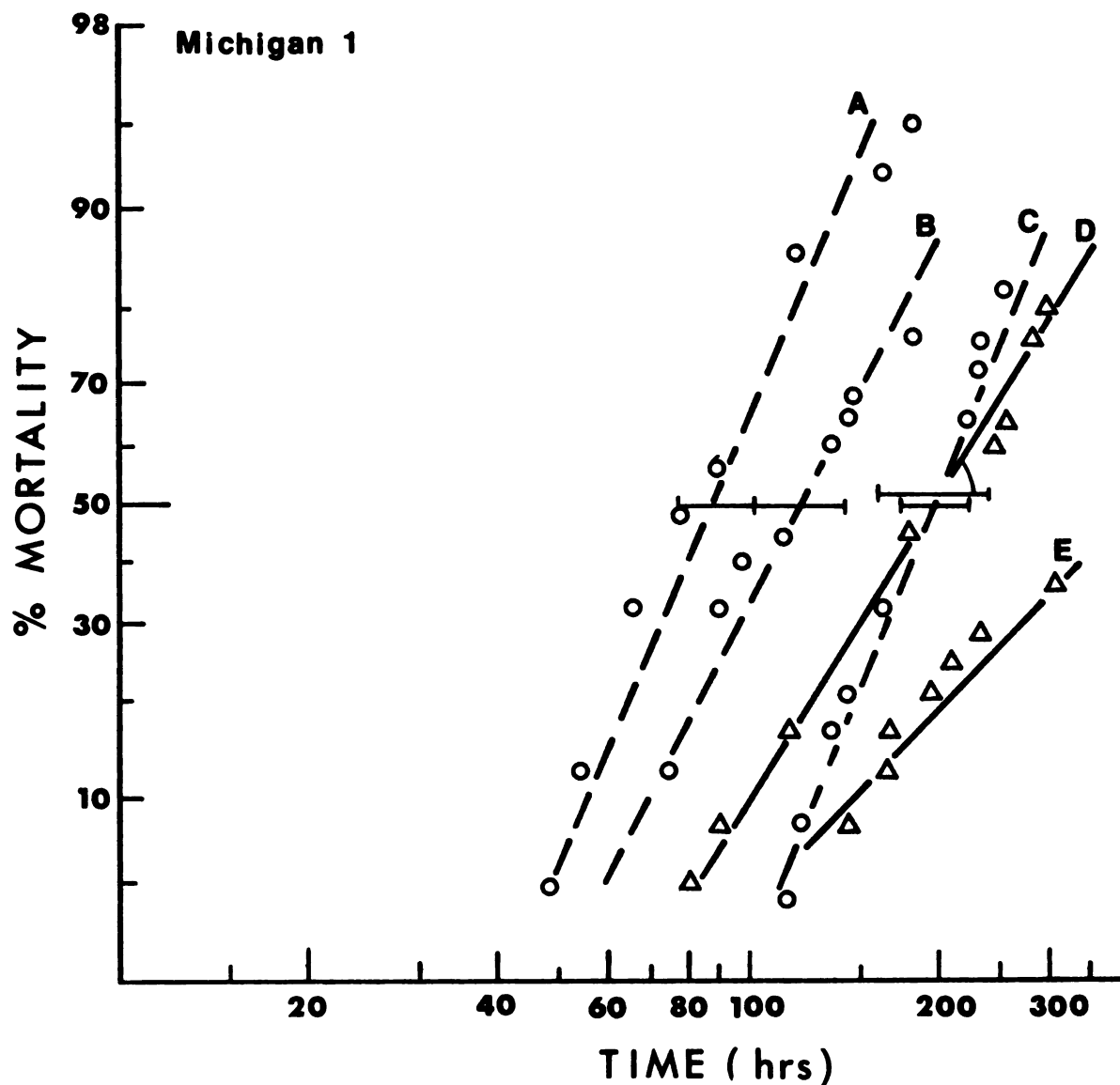
^aApproximate toxicant concentrations. Actual concentrations not measured.

Table 5. Summary of toxicity data for Oregon coho salmon exposed to various concentrations of PCB, DDT or PCB-DDT combinations.

Test No.	Fish Data		Test Conc.		Median Survival Times (MST) (hours)	95% Confidence Interval (hours)	
	Start	Finish	PCB	DDT			
3	\bar{X} length (mm)	38	45	--	2.87	25	22-28
	\bar{X} weight (gm)	0.43	0.80	40.90	+2.72	42	36-49
				--	1.40	55	49-62
				20.75	+1.25	70	61-81
3.5				11.70	+0.80*	108*	86-136
				--	0.82*	125*	
				--	3.29	25	23-27
	\bar{X} length (mm)	45	47	44.00	+2.15*	54*	48-61
	\bar{X} weight (gm)	0.80	0.88	--	1.90*	55*	47-65
				25.15	+1.15	99	86-114
				12.80	+0.60	> 168	----
				--	0.72	> 168	----
				33.00	+2.00	172	140-211
				15.25	+0.80	336	----
4	\bar{X} length (mm)	47	54	32.20		336	----
	\bar{X} weight (gm)	0.88	1.40	9.80	+0.45	336	----
				16.50		336	----

*These test concentrations or median survival times are not significantly different at $p = .05$.

Figure 3. The toxicity of various concentrations of PCB and PCB-DDT combinations to young Michigan coho salmon. Broken lines represent PCB-DDT toxicity, solid lines PCB toxicity. Confidence interval (95%) of the median survival times are indicated by horizontal bars.

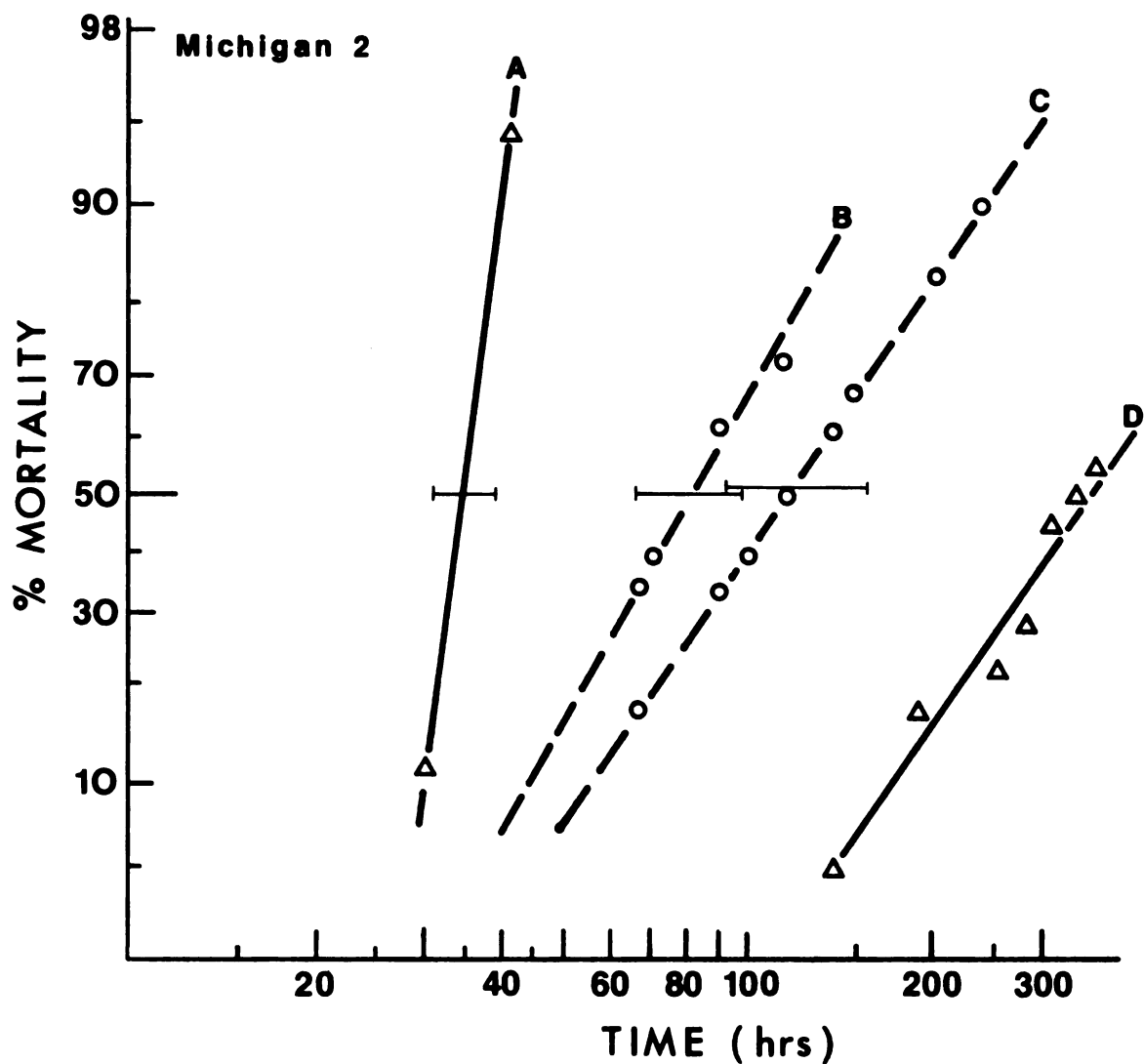


MEDIAN SURVIVAL TIMES

<u>Line</u>	<u>Conc. (ppb)</u>		<u>MST</u>
	<u>PCB</u>	<u>DDT</u>	
A	41.00	+ 2.75	88
B	20.00	+ 1.50	120
C	10.20	+ .65	200
D	47.60	—	196
E	28.25	—	> 336

Figure 3

Figure 4. The toxicity of various concentrations of DDT and PCB-DDT combinations to young Michigan coho salmon. Broken lines represent PCB-DDT toxicity, solid lines DDT toxicity. Confidence interval (95%) of the median survival times are indicated by horizontal bars.

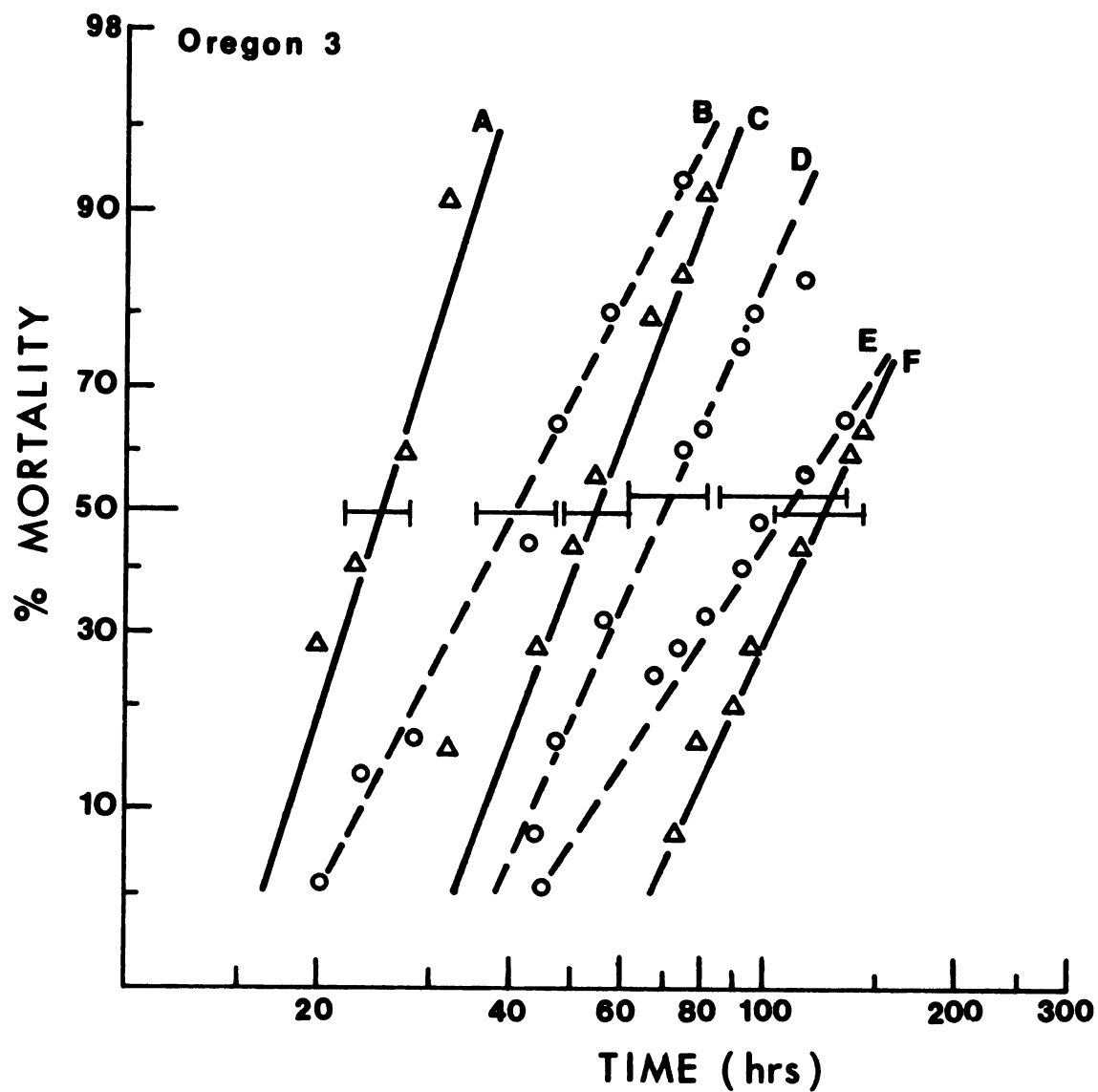


MEDIAN SURVIVAL TIMES

<u>Line</u>	<u>Conc. (ppb)</u>		<u>MST</u>
	<u>PCB</u>	<u>DDT</u>	
A	—	3.10	34.5
B	44.50	+ 2.80	80.0
C	24.00	+ 1.50	115.0
D	—	.95	>330

Figure 4

Figure 5. The toxicity of various concentrations of DDT and PCB-DDT combinations to young Oregon coho salmon. Broken lines represent PCB-DDT toxicity, solid lines DDT toxicity. Confidence interval (95%) of the median survival times are indicated by horizontal bars.

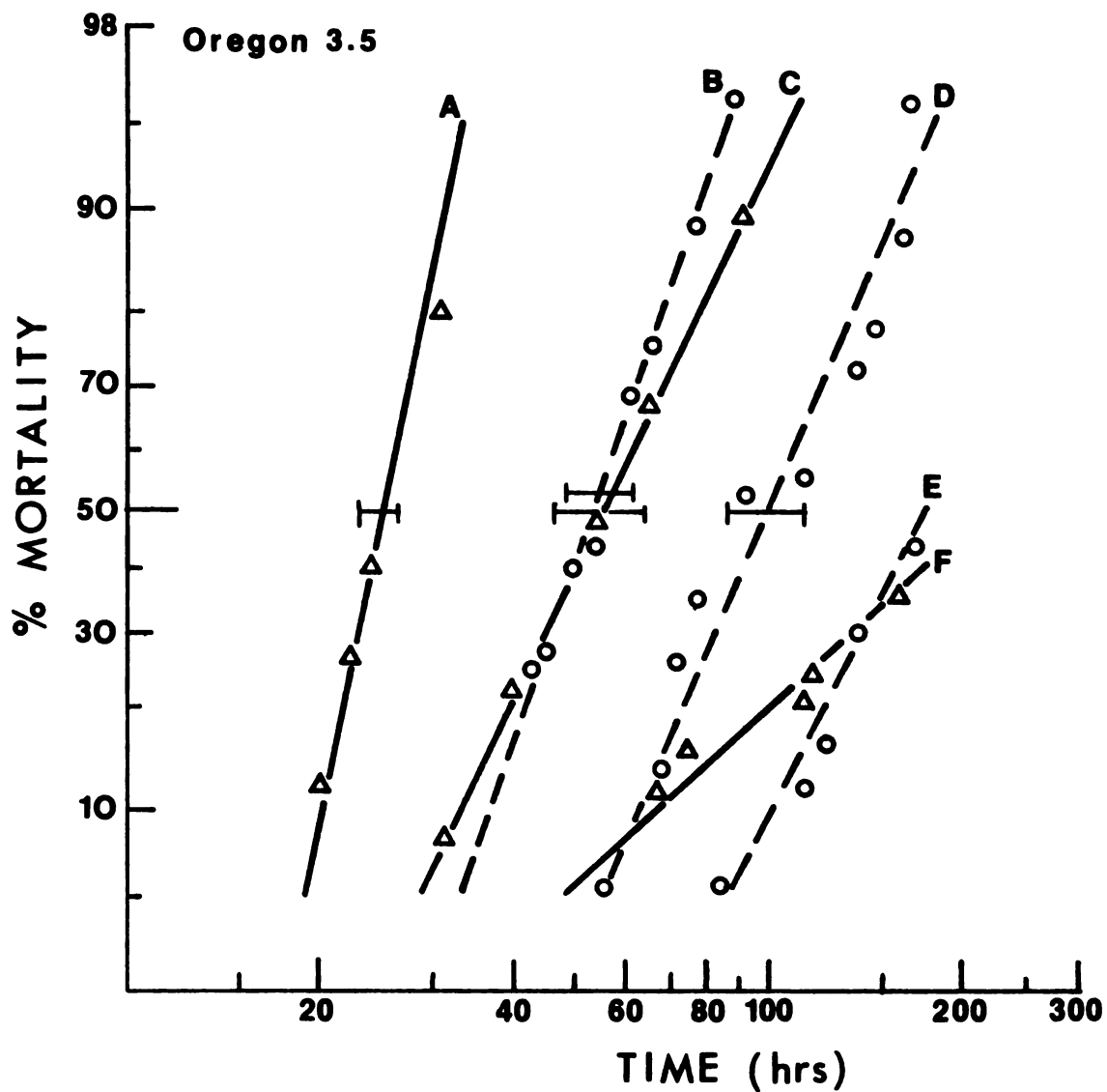


MEDIAN SURVIVAL TIMES

<u>Line</u>	<u>Conc. (ppb)</u>		<u>MST</u>
	<u>PCB</u>	<u>DDT</u>	
A	—	2.87(3.20)	25
B	40.90	+ 2.72	42
C	—	1.40	55
D	20.75	+ 1.25	70
E	11.70	+ .80	108
F	—	.82	125

Figure 5

Figure 6. The toxicity of various concentrations of DDT and PCB-DDT combinations to young Oregon coho salmon. Broken lines represent PCB-DDT toxicity, solid lines DDT toxicity. Confidence interval (95%) of the median survival times are indicated by horizontal bars.

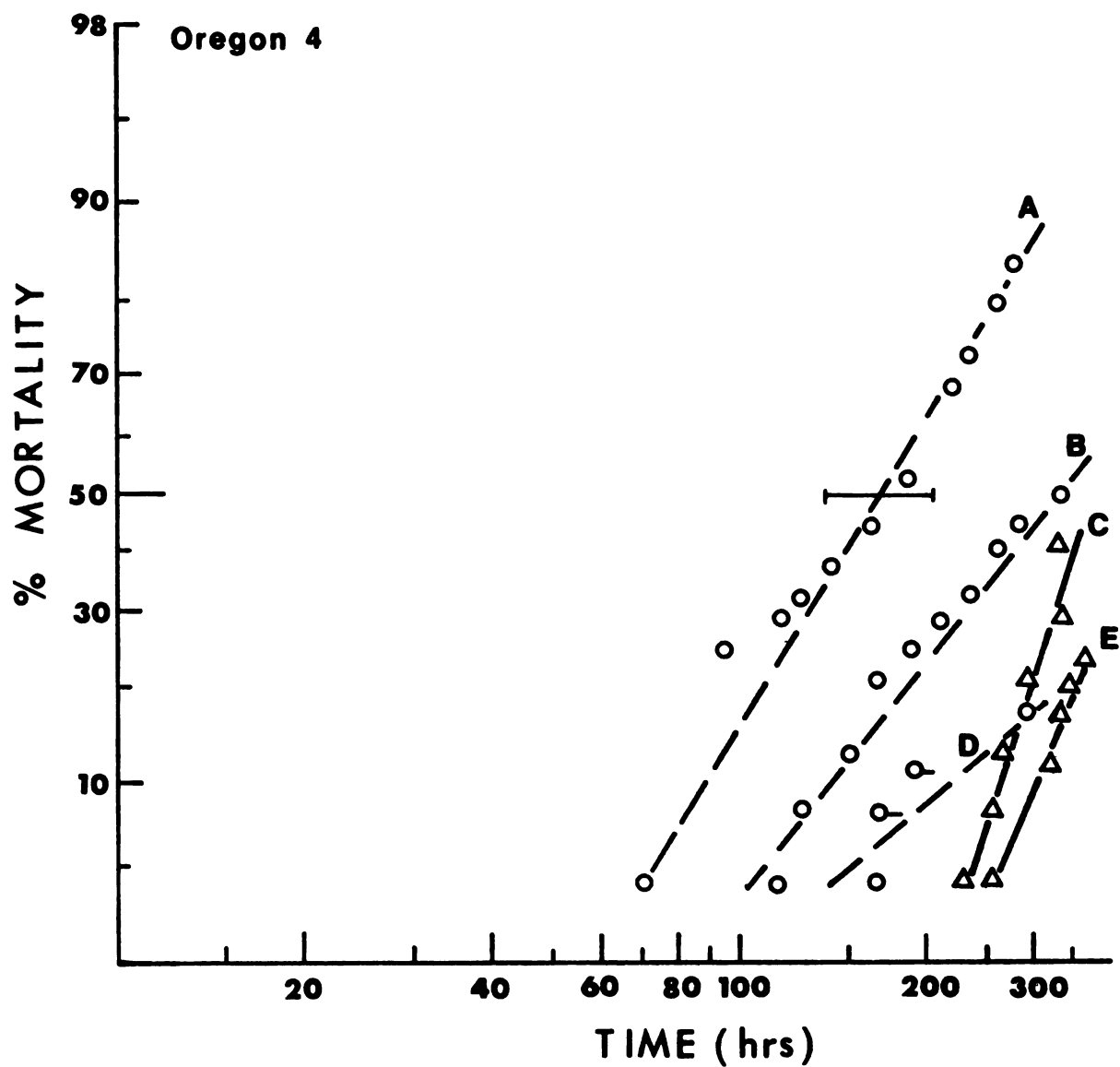


MEDIAN SURVIVAL TIMES

<u>Line</u>	<u>Conc. (ppb)</u>		<u>MSI</u>
	<u>PCB</u>	<u>DDT</u>	
A	—	3.29	25
B	44.00	+ 2.15	54
C	—	1.90	55
D	24.15	+ 1.15	99
E	12.80	+ .60	> 168
F	—	.72	> 168

Figure 6

Figure 7. The toxicity of various concentrations of PCB and PCB-DDT combinations to young Oregon coho salmon. Broken lines represent PCB-DDT toxicity, solid lines DDT toxicity. Confidence interval (95%) of the median survival times are indicated by horizontal bars.



MEDIAN SURVIVAL TIMES

<u>Line</u>	Conc. (ppb)		<u>MST</u>
	<u>PCB</u>	<u>DDT</u>	
A	33.00	+ 2.00	172
B	15.25	+ .80	> 336
C	32.20	—	> 336
D	9.80	+ .45	> 336
E	16.50	—	> 336

Figure 7

Johnson and Pecor (1969). This mortality was not unexpected and the timing and symptoms were identical to that observed among Michigan salmon in previous years. The mortality occurred while the first bioassay with Michigan salmon was in progress and some losses due to this mortality occurred among the test fish. However the symptoms associated with these losses were distinct from those induced by the toxicants in the bioassays, and were only evident during the last 100 hours of the test. In the PCB exposure system 9, 5, and 2 fish died in the control and two lowest PCB concentrations, respectively, while the same tanks of the PCB-DDT combination system had 4, 5, and 4 killed, respectively. These mortalities occurred after 200 hours into the test and are not shown in the graphs of the test results. The stock tank mortality had ceased before the second test was initiated and no losses occurred in the controls or two lowest test concentrations during this test. The median survival times for fish in the higher PCB-DDT mixtures of the second test compare favorably with those of the first test.

There was a marked difference in symptoms between PCB and DDT poisoned salmon. Fish exposed to 40 µg/l Aroclor 1254 became hypoactive and ceased feeding after about three days. They oriented near the bottom of the tank, finning only enough to maintain their position in the water. Darkening in color occurred in some fish but not in all. The fish did not readily respond to a pencil tap on the aquarium frame,

whereas control fish reacted by darting around the tank. About a day before death, the fish swam listlessly near the surface of the water and did not react to a prod with a glass rod. Death was preceded by a several hour period of slow, deep operculating by the fish on the bottom of the aquarium.

No external lesions appeared on fish exposed to PCB. In other studies, Hansen et al. (1970) and Morgan (1972) have reported the development of external lesions on spot (Leiostomus xanthurus) and guppies (Poecilia reticulata), respectively, exposed to Aroclors 1254 and 1242, respectively.

Fish exposed to about 2 µg/l DDT became hyperactive within a day after beginning exposure. A pencil tap on the aquarium frame sent fish darting in all directions with more intensity and for longer duration than did a similar tap on the control tank. Moribund fish lost equilibrium, convulsed periodically, and died, often with mouth open and back arched. The time of death after appearance of acute symptoms was typically less than a day, always less than two days. Fish killed in the PCB-DDT combinations showed the symptoms of DDT poisoning.

The general mortality among the Michigan coho which occurred at the final yolk-sac absorption stage had some of the features of both PCB and DDT toxicity. Darkening occurred in some of the fish. Swimming patterns were erratic and convulsive for a time, but the fish then sank and remained on

the bottom for four to five days before dying. There was no evidence that dying fish had initiated feeding behavior.

Comparison of the length and weight of control fish with fish tested in the two lowest toxicant concentrations in each of the bioassays indicated no consistent effect of PCB or DDT on growth over the two week periods. Growth measurements were not obtained for fish in the higher test concentrations because of partial or complete mortality.

Discussion

The bioassay results indicate that the PCB-DDT concentrations caused no greater mortality than could be expected from exposure to the DDT concentration alone. We conclude that PCB does not increase the toxicity of DDT to young coho salmon at the acute toxicity level, although both compounds are quite toxic in themselves. The lack of interaction between PCB and DDT may be explained by the difference in their modes of action to fish. DDT kills rapidly whereas PCB causes a more chronic toxicity. DDT is most likely a direct neurotoxin (O'Brien, 1967) while the mode of action of PCB is not yet clear. Recent studies have shown that both DDT and PCB inhibit the ATP-ase enzyme system in fish (Cutkomp et al., 1971; Davis and Wedemeyer, 1971; Koch et al., 1972). In our experiments high DDT concentrations were always mixed with high PCB concentrations and the faster-acting DDT always

killed the test fish before any toxicity due to PCB could be expressed. To more completely evaluate PCB-DDT interactions, tests of high PCB-low DDT should be conducted.

COHO SALMON EGG-ALEVIN EXPOSURE

Methods and Materials

The purpose of this test was to determine the effect of Aroclor 1254 exposure on the embryo and alevin stages of coho salmon. A proportional diluter equipped with a 1.0 ml toxicant introduction system delivered a water control and five concentrations of Aroclor 1254. A second identical system delivered a water control and five concentrations of acetone in the same amounts as the first system. Acetone was not delivered to the control tanks.

On November 24, 1971, about 100 eyed Lake Michigan coho salmon eggs were placed onto the bottom of each of the PCB exposure chambers. Two weeks later, and about two days prior to hatching, half of each egg group was transferred to the appropriate chamber of the uncontaminated system. Egg hatching success and alevin survival were then monitored in both systems until January 15, 1972, four weeks beyond hatching. The test chambers were darkened by black plastic curtains until the fry began to swim-up, then laboratory light was admitted into portions of the tanks. At the end of the test, surviving fry were sacrificed and a subsample of twenty fish, if available, from each test chamber were weighed and

measured. Mean lengths and weights were compared by the Student t-test at $p = 0.05$.

One liter water samples were taken from each tank of the PCB exposure system every five days during the test for actual toxicant concentration determination.

Results

The hatchability of salmon eggs continuously exposed to 56.40 $\mu\text{g/l}$ Aroclor 1254 (Table 6 and Appendix A) was reduced by 30% compared to the control eggs. Variable hatching success was observed at the lower PCB levels. At hatching, about half of the losses occurred as the alevins emerged from the egg and the remainder died within the intact egg. In the four weeks following hatching, alevin survival was inversely related to the PCB concentration, with no group surviving as well as the control. Fry exposed to greater than 15 $\mu\text{g/l}$ PCB absorbed their yolk sacs and grew slower than the control fry.

Salmon eggs removed from PCB exposure prior to hatching (Table 7 and Appendix A) showed good hatchability (90-97%), except those which had been exposed to 56.40 $\mu\text{g/l}$. The latter eggs sustained a 27% reduction compared to the controls. Alevins from egg groups which had been exposed to greater

Table 6. Summary of test in which eyed coho salmon eggs were incubated, hatched, and the resulting alevins allowed to develop for four weeks in various concentrations of Aroclor 1254.

Nominal PCB conc. ($\mu\text{g/l}$)	Measured \pm SD	Percent eggs hatched	Mean incub. time (de-gee-days)	Percent fry survival	Fry data measured at end of test			
					\bar{X} length \pm SD (mm)	\bar{X} weight \pm SD (g)	n	Yolk sac utilization ^a
0	0	95.5	511	91.0	35.95 \pm 0.89	.30 \pm .03	20	0
5	4.35 \pm 0.63	88.0	485	75.5	34.65 \pm 1.27*	.30 \pm .02	20	0
10	7.75 \pm 1.89	78.5	485	64.0	34.40 \pm 1.00*	.30 \pm .06	20	.5
20	15.35 \pm 1.33	38.0 ^b	---	24.0	31.92 \pm 1.88*	.27 \pm .03*	12	1
40	25.90 \pm 3.16	96.5	459	6.8	29.75 \pm 2.22*	.28 \pm .01	4	2
80	56.40 \pm 9.23	63.0	462	6.5	28.33 \pm 0.58*	.24 \pm .04*	3	3

^aRated on a scale of 0-6. 0 = button-up stage, 6 = complete yolk sac.

^bBacteria or fungi caused excessive mortality of eggs.

* Significantly different from control fish at $p = .05$.

Table 7. Summary of test in which eyed coho salmon eggs were incubated for two weeks in various concentrations of Aroclor 1254, then transferred to an uncontaminated system for hatching and development.

Eggs removed from PCB conc.: (µg/l)	Percent eggs hatched	Mean incub. time (de-gee-days)	Percent fry survival	Fry data measured at end of test		
				\bar{X} length+SD	\bar{X} weight+SD	Yolk sac utilization ^a
0	93.0	503	93.0	35.30+0.98	.30+ $\underline{.03}$	20 0
4.35	95.5	475	69.5	35.20+0.89	.30+ $\underline{.02}$	20 0
7.75	90.0	490	88.0	34.70+1.03*	.30+ $\underline{.02}$	20 0
15.35	91.5	490	83.5	33.25+1.37*	.26+ $\underline{.03}$ *	20 1.5
25.90	97.5	481	74.5	34.45+1.32*	.29+ $\underline{.02}$	20 1
56.40	66.0	488	39.5	33.73+1.71*	.26+ $\underline{.04}$ *	15 2

38

^aRated on a scale of 0-6. 0 = button-up stage, 6 = complete yolk sac.

* Significantly different from control fish at p = .05.

than 15 µg/l PCB had significantly reduced survival, yolk sac utilization and growth, but generally did better than those fry held under continuous exposure to these concentrations in the other system. The alevins from eggs which had been exposed to less than 8 µg/l PCB did as well as the controls, except as discussed below.

Eggs in both exposure systems started hatching two to five days before the control eggs so that mean incubation times were reduced (Tables 6 and 7). This early hatching was preceded by an alteration of the egg surface which appeared to be due to a breakdown or coagulation of the outer surface of the chorion. The eggs continuously exposed to greater than 25 µg/l PCB hatched most prematurely, about 50 degree-days or four days before the controls.

Discussion

Under continuous exposure to greater than 15 µg/l Aroclor 1254, coho salmon eggs and alevins showed marked reductions in survival and growth. This was true even when exposure was removed prior to egg hatching, although removal did somewhat alleviate these effects. In the latter case, the detrimental effects were probably due to the toxic action of PCB absorbed by the embryo through the egg chorion during exposure. Toxicity may have been enhanced by stresses acting upon prematurely hatched alevins. Acetone was also present in the test system, but is not believed to be an important

factor in the results. Separate tests with coho eggs and fry in our laboratory (unpublished data) showed that acetone concentrations of 800 mg/l had no effect on the survival of fry. Still it may be argued that acetone could serve as a predisposing or facilitating agent for the action of known toxicants, such as PCB.

Eggs and alevins continuously exposed to even the lowest PCB level tested here (4.35 $\mu\text{g/l}$) had significant reductions in at least three of the parameters measured. On the other hand, the alevins from eggs removed from less than 8 $\mu\text{g/l}$ showed no overall effects of the PCB treatment, except for premature hatching. The significance of these findings with respect to successful long-term fish production can only be evaluated on the basis of longer tests.

Jensen et al. (1970) have correlated a mortality of Swedish salmon embryos (eggs) with PCB residues in the eggs of 7.7-34.0 $\mu\text{g/g}$ (fat basis). The severity of losses was directly related to the amount of residues in the egg lots. Eggs taken from adult fish reared in freshwater had higher PCB residues and mortality than eggs taken from adults reared in the sea. Our results in part indicate that PCB is capable of killing salmon embryos within the egg and thus support Jensen's data implicating PCB in egg losses.

PCB UPTAKE BY STEELHEAD TROUT

Methods and Materials

This investigation measured the rates of PCB accumulation in young steelhead trout exposed for 24 days to five concentrations of Aroclor 1254 (3.25-51.30 $\mu\text{g/l}$). The test concentrations were delivered by a proportional diluter equipped with a 1 ml toxicant introduction system. Acetone was not delivered to the control tank.

On July 13, 1971, fifty fish (avg. weight 0.35 g, avg. length 36 mm, 60 days old) were randomly assigned to each test chamber. The daily test routine was as described earlier for the PCB-DDT combination bioassays. As fish died, brains (and the brain case) were excised and placed in individual aluminum foil envelopes. The bodies were placed in similar packets and both were then frozen. On days 5, 10, and 16 of the test, four live fish, apparently in good condition, were removed from each test tank, sacrificed, and processed in the same manner. One liter water samples were taken from each tank every five days for determination of actual PCB exposure concentrations (Table 8).

Fish brain and body tissues were extracted by maceration in a tissue grinder with several portions of hexane which had

Table 8. The Aroclor 1254 concentrations, in $\mu\text{g}/\text{l}$, present in the test tanks during the PCB uptake study as determined by gas chromatography.

Nominal	Measured \pm SD	SE	Range	No. of analyses
0	---	---	---	6
5	3.25 \pm 0.16	0.080	3.01- 3.41	5
10	6.15 \pm 0.32	0.161	5.22- 7.80	5
20	10.45 \pm 3.39*	1.517	6.00-14.12	6
40	27.80 \pm 4.11	1.840	21.94-31.96	6
80	51.30 \pm 6.66	2.980	41.79-60.64	6

*Diluter delivered only about half of the desired amount of toxicant to this test chamber on days 7 through 11 of this test.

been heated to 60°C. The extraction efficiency for this method was not determined, but repeated extractions beyond the normal end point in more than 10 trials yielded no additional residues. Extracts were concentrated under a gentle stream of nitrogen or by rotary evaporator and transferred to a graduated centrifuge tube before gas chromatographic analysis. The GLC conditions were the same as those described for water analysis in the general methods. Clean-up procedures were found to be unnecessary. The heights of peaks 4 through 9 of Aroclor 1254 (Figure 2) were again used for quantitation although some alteration of the relative heights of peaks 4, 8, and 9 occurred in these tissue samples (Figure 8).

The stock fish contained 1-2 µg/g chlorinated hydrocarbon residues (Johnson, 1972), but these amounts were insignificant compared to the levels of PCB accumulated during the exposure period.

The PCB concentrations in live and dead fish were pooled to calculate linear regression lines describing the uptake rates of PCB, since examination of the raw data points indicated no difference in their residue concentration.

Results

The uptake of Aroclor 1254 by steelhead trout based on whole body residues (Figure 9) was dependent on time of exposure and exposure concentration. Uptake was linear over the time period monitored, from 100 to 576 hours.

Figure 8. A comparison of standard and tissue sample chromatograms of Aroclor 1254. In tissue sample chromatograms the relative heights of peaks 4, 8, and 9 were altered in the manner shown.

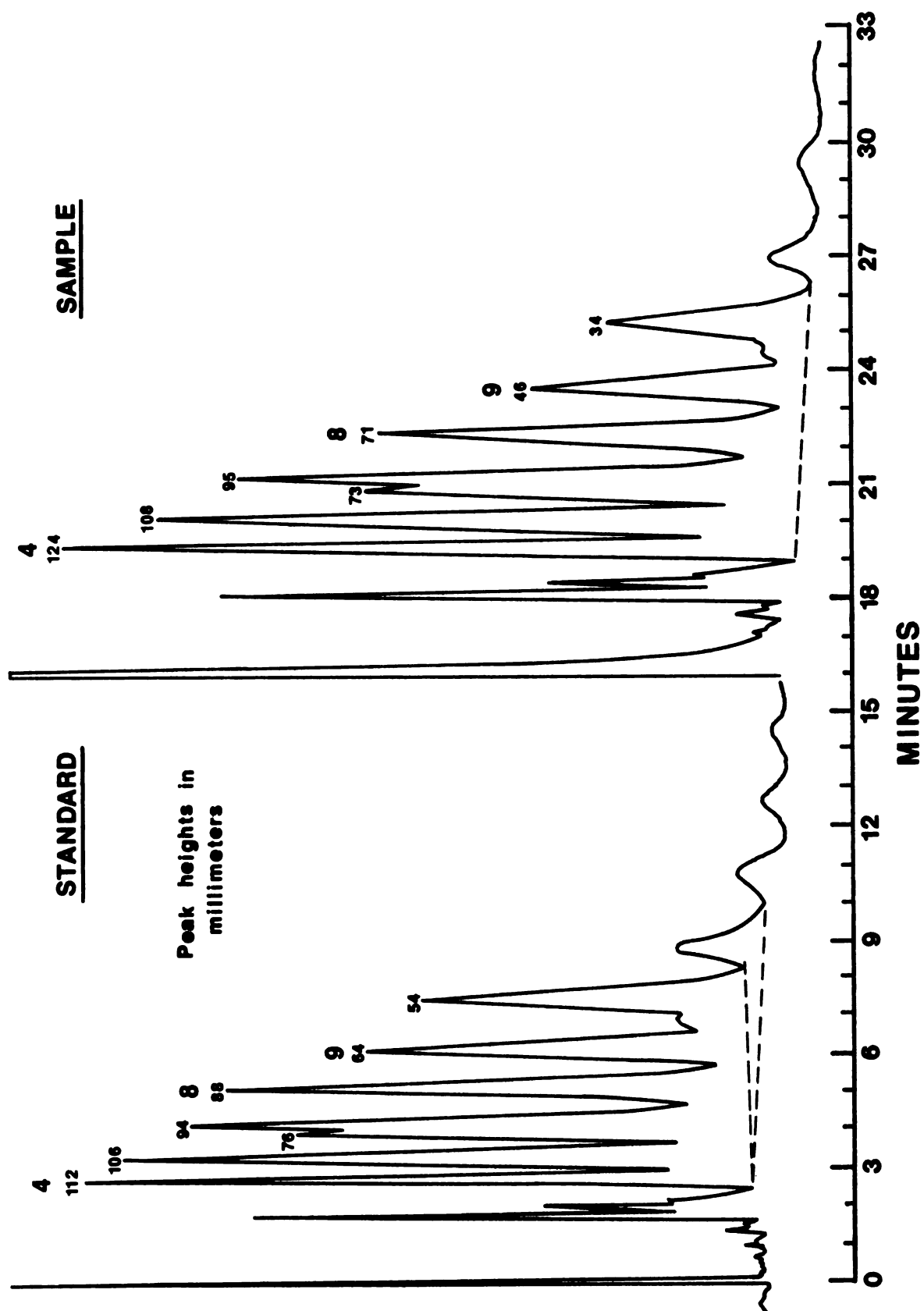


Figure 8

Figure 9. The uptake of Aroclor 1254 from water by young steelhead trout. The exposure concentration is given at the upper end of each regression line. Open triangles and dots represent fish exposed to 51.30 or 10.45 $\mu\text{g/l}$ PCB. Solid triangles and dots represent fish exposed to 27.80 or 3.25 $\mu\text{g/l}$ PCB.

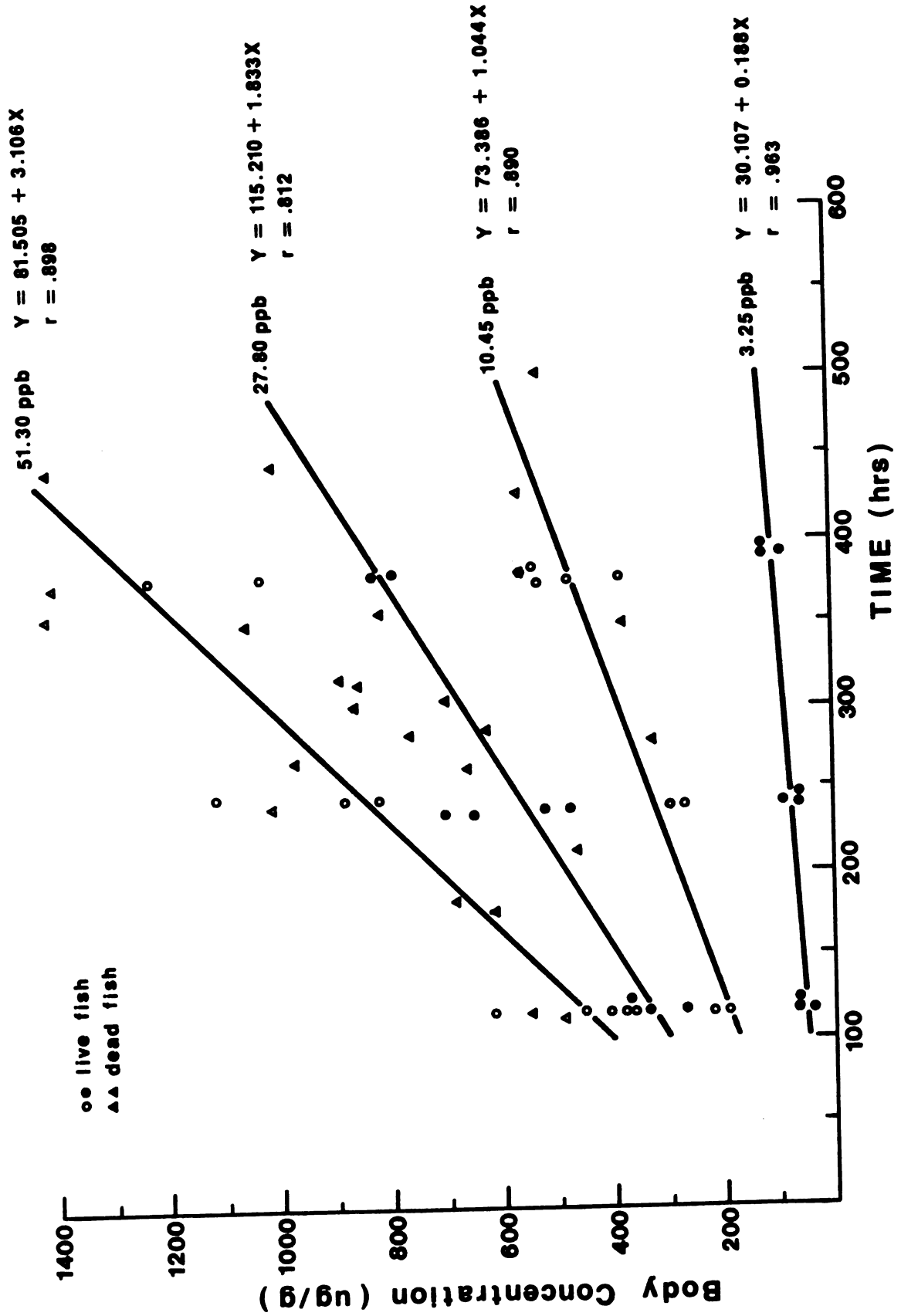


Figure 9

The rate of PCB uptake in $\mu\text{g/g}$ per hour from each test concentration is given by the slope of the regression lines in Figure 10. The rates were 3.106, 1.832, 1.044 and 0.189 $\mu\text{g/g}$ per hour from the highest to lowest exposure concentration, respectively. To determine whether uptake rates could be predicted if an uptake rate from a specific PCB concentration was known, the simple equation:

$$\frac{\text{test concentration "X"}}{\text{known concentration "Y"}} \times \text{known uptake rate from "Y"} = \text{predicted uptake rate from "X"}$$

was used to predict uptake rates from the experimental concentrations. The data of the highest PCB level (51.30 $\mu\text{g/l}$, 3.106 $\mu\text{g/g}$ per hour) was selected as the "known" information and the following values were then calculated:

"X" ($\mu\text{g/l}$)	UPTAKE VALUES ($\mu\text{g/g}$ per hour)	
	<u>Predicted</u>	<u>Actual</u>
27.80	1.680	1.832
10.45	0.633	1.044
3.25	0.195	0.189

Within experimental limits the predicted uptake rates are good. A diluter malfunction in the delivery of toxicant to the test chamber of 10.45 $\mu\text{g/l}$ is responsible for the discrepancy between those values (see Table 8).

The degree to which Aroclor 1254 was concentrated in the fish tissues over the exposure concentration at 100 hour

Figure 10. Aroclor 1254 concentrations in the bodies and brains of young steelhead trout killed by exposure to 51.30 µg/l Aroclor 1254.

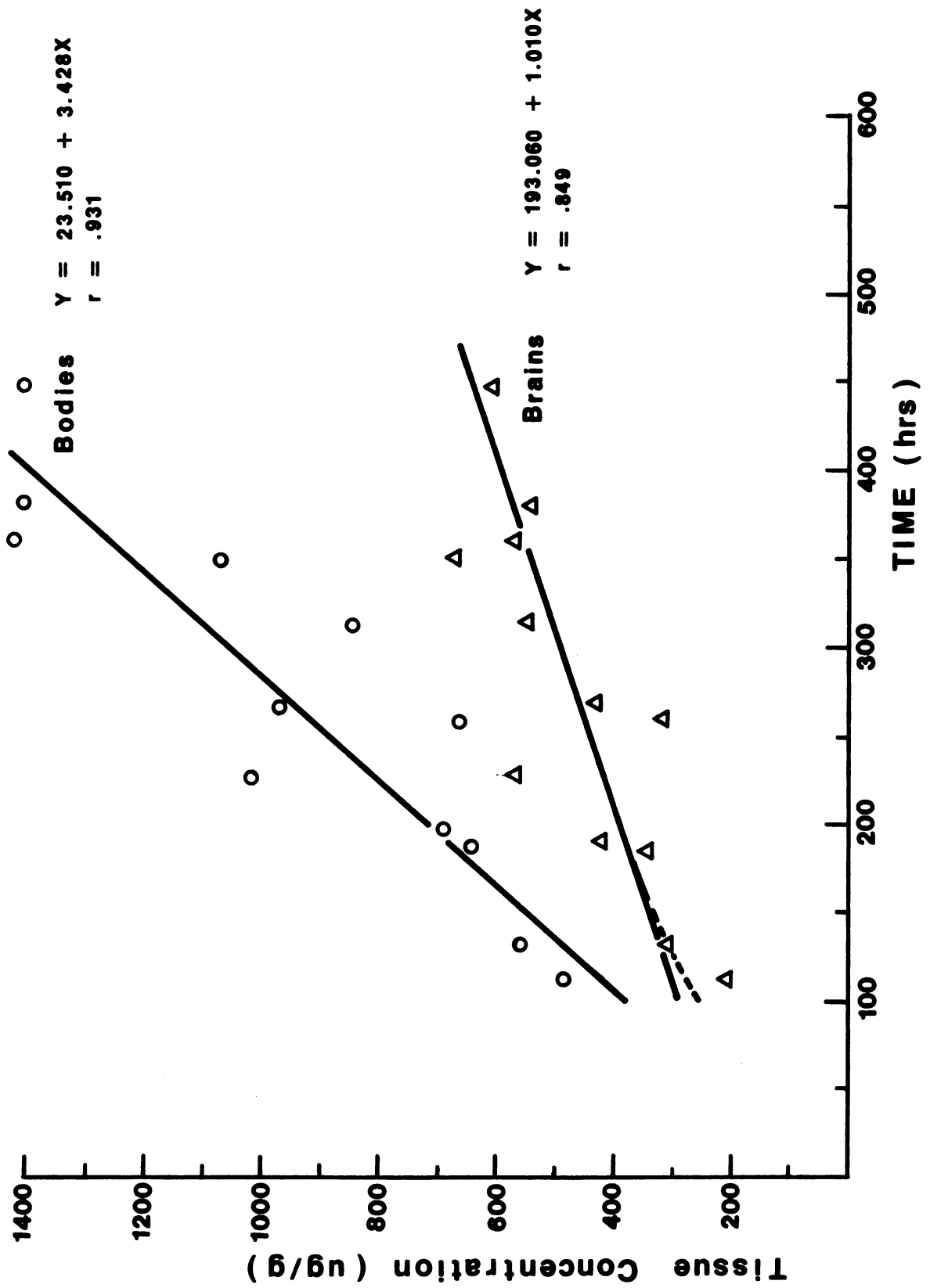


Figure 10

intervals was calculated using the regression equation of each line. Whole body residue concentrations derived by substituting the appropriate number of hours into each equation were divided by the exposure concentration to obtain magnification factors (Table 9). The factors increase with time because uptake and exposure were continuous over the test period. The reason for the inverse relationship between exposure concentration and the calculated magnification factors is not immediately clear however since uptake rate declined in direct proportion to exposure concentration, as shown above. In fact, when concentration factors are calculated from the regression equations excluding the Y-intercept values, then the resultant factors (Table 10) show that concentration occurs fairly uniformly over all test levels at about 6000X increments per 100 hours. The magnitude of the actual concentration factors is therefore related to uptake phenomena occurring before 100 hours in the exposure period when uptake has not yet become linear. During this time it appears that fish exposed to low PCB concentrations take up relatively more PCB than do the fish in the higher PCB concentrations. This process stops sometime before 100 hours of exposure but the effect on bioconcentration is still evident in this test at 576 hours, when exposure ended.

Residue concentrations in the bodies and brains of fish killed by exposure to 51.30 $\mu\text{g/l}$ Aroclor 1254 are shown in Figure 10. These data suggest that after 200 hours exposure,

Table 9. Actual magnification factors calculated by substitution of the appropriate number of hours into the regression equations describing PCB uptake.

		Time (hours)				
		100	200	300	400	500
Exposure	51.30	7,600	13,700	19,700	25,800	31,800
Concentration	27.80	10,700	17,300	23,900	30,500	37,100
($\mu\text{g/l}$)	3.25	15,200	21,000	26,800	32,000	38,500

Table 10. Magnification factors calculated by substitution of the appropriate number of hours into the regression equations, excluding the "Y" intercept value, describing PCB uptake.

		Time (hours)				
		100	200	300	400	500
Exposure	51.30	6,050	12,100	18,150	24,200	30,250
Concentration	27.80	6,600	13,200	19,800	26,350	32,950
($\mu\text{g/l}$)	3.25	5,850	11,700	17,500	23,400	29,200

or after accumulating about 400 $\mu\text{g/g}$, the further accumulation of PCB in the brain lags behind whole body uptake.

Discussion

The PCB exposure concentrations of this study were selected to insure that fish in the higher levels would die during the course of the experiment, since the original purpose of this study was to compare the brain concentrations of PCB in live and dead fish from the same exposure concentration, such as was done with dieldrin and bluegills by Hogan and Roelofs (1971). During the progress of the work however, the brains of the sacrificed fish were improperly extracted and brain data was obtained from dead fish only. The bodies of the test fish were therefore analyzed and are presented here as an uptake experiment. But when the purpose of the test shifted, the exposure concentrations became too high to present an uptake picture that is environmentally significant. Nonetheless, the data do provide a quantitative demonstration of the accumulation of a hydrophobic chlorinated hydrocarbon from water by fish. The apparent greater efficiency of uptake early in the test in the lower concentrations evident here has appeared in the work of others, was mentioned by Chadwick and Shumway (1969), but has not been discussed. Concentration factors calculated from the PCB data of Duke et al. (1970, Table II, p. 179), the dieldrin data of Chadwick and Shumway (1969, Figure 4, p. 93) or Chadwick and Brocksen

(1969, Figure 2, p. 696), and the parathion-blood data of Mount and Boyle (1967, Figure 2, p. 1185) are highest in fish exposed to the lowest concentrations.

A definite explanation for this phenomenon is, to our knowledge, lacking. However, a hypothesis may be formulated based on the work of P. O. Fromm, Michigan State University. First it is assumed that the primary site of uptake of toxicants into fish is at the gills (Holden, 1962; Premdas and Anderson, 1963; Fromm and Hunter, 1969). Fromm et al. (1971) have shown that the rate of fluid flow through isolated-perfused rainbow trout gills is decreased when 1 mg/l dieldrin, or several other chemicals, is added to the perfusion medium. Earlier these authors (Richards and Fromm, 1969) found that the addition of epinephrine to the perfusion fluid caused an increase in the overall flow rate through the gills. It is possible then that fish exposed to various concentrations of toxicants in uptake studies have gill perfusion rates decreased in direct proportion to the concentration of toxicant in the exposure system. This would allow the fish exposed to lower concentrations to accumulate relatively more chemical than the fish in the higher concentrations. Perhaps then, after a time, the fish in the higher concentrations make a physiological adaptation in the form of an increased hormone secretion and the gill perfusion rate returns to a more normal level.

Alternatively, it may be argued that the reduction in gill perfusion rates occurs and persists in fish exposed at all PCB levels for as long as the exposure is continued. The effect would occur most rapidly in those fish in the highest PCB concentration and slowest in the lowest concentration. But all fish would eventually show the same degree of gill perfusion reduction. For a time however the fish in the lower PCB concentrations would accumulate PCB at a relatively higher rate than the fish in the higher concentrations. Either version of this hypothesis would account for the observed effects in uptake studies, but both are obviously only speculation.

Our overall results are similar to the findings of Chadwick and Brocksen (1969) who studied accumulation of dieldrin from water by sculpin. The chief difference between uptake of dieldrin in sculpin and PCB in steelhead appears to be in magnitude, with PCB being more readily concentrated.

The highest bioconcentration factors measured in this study were about 32,000-38,000 and these were still increasing at the conclusion of the exposure. Hansen et al. (1971) found that spot (Leiostomus xanthurus) accumulated Aroclor 1254 37,000 times over the 1 $\mu\text{g}/\text{l}$ exposure concentration in 28 days and that this was about the plateau of the concentration factors. Preliminary results reported by Stallings and Mayer (1972) indicate that bluegills concentrated Aroclor 1254 26,000-71,000 times from water concentrations of 2-10 $\mu\text{g}/\text{l}$ in

an 11 week exposure period but the authors did not state whether these were plateau levels. They also reported that PCB is not rapidly taken up from contaminated food by coho salmon which suggests that water contamination may be the major source of PCB residues in fish.

STEELHEAD TROUT TOXICITY BIOASSAY

Methods and Materials

The same PCB exposure system as described for the uptake experiment was used to conduct a thirty day toxicity bioassay with young steelhead trout. At the start of the test, 240 fish (avg. length 47.6 mm, avg. weight 1.07 gm, 100 days old) were anesthetized in a 50 ml/l solution of MS-222, weighed, measured, and allowed to recover in fresh water for one hour before introduction into the test chambers. Forty fish per concentration were tested. The daily bioassay routine was as described for the PCB-DDT combination tests. After thirty days of exposure, surviving fish were sacrificed and re-measured.

Water samples were taken from each test chamber every five days for actual toxicant concentration determination (Table 11).

Median survival times (MST) and their 95% confidence limits were calculated according to Litchfield (1949) and mean lengths were compared by the Student t-test ($p = 0.05$). The test was not replicated but the sample size ($n = 40$) tended to minimize the relative error of the calculated MST values (Jensen, 1972).

Table 11. The Aroclor 1254 concentrations, in $\mu\text{g/l}$, present in the test tanks during the thirty day toxicity test as determined by gas chromatography.

Nominal	Measured \pm SD	SE	Range	No. of analyses
0	0	---	---	6
5	2.55 \pm 0.18	0.081	1.93- 3.47	6
10	4.75 \pm 1.57	0.702	2.52- 6.66	6
20	10.15 \pm 2.09	0.935	8.21-13.33	6
40	20.40 \pm 3.34	1.492	16.05-26.12	6
80	39.40 \pm 5.42	2.422	32.63-47.61	6

Results

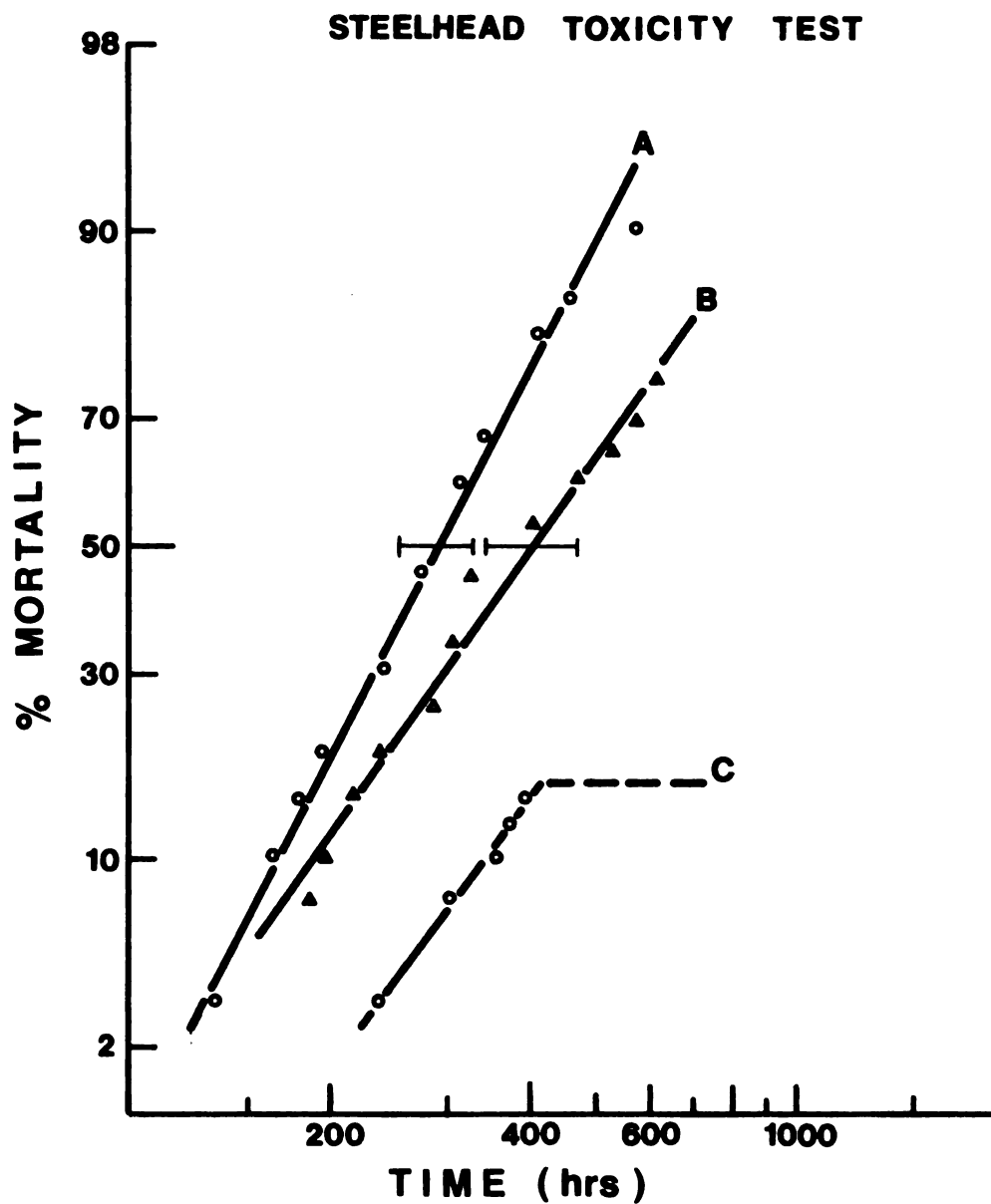
Cumulative percentages of dead fish plotted on probability scale against time to death (Figure 11) show that 39.40 and 20.40 $\mu\text{g/l}$ Aroclor 1254 killed half of the test fish in 293 and 400 hours (12.2 and 16.6 days), respectively. Only six fish died in 10.15 $\mu\text{g/l}$ PCB and none died in the two lower concentrations or the control. Dying fish displayed the symptoms of PCB poisoning described in the PCB-DDT bioassay section which include loss of appetite, listlessness, and slow death.

Fish growth as measured by length was significantly reduced by exposure to greater than 4.70 $\mu\text{g/l}$ PCB (Table 12). Differences in the weight of the fish could not be statistically tested since fish were initially weighed in small groups to minimize handling stress and standard error estimates were thus not available. However, the average weight of fish exposed to PCB showed obvious declines as the test concentrations increased.

Discussion

The chronic nature of PCB toxicity is evident from these bioassay results. The thirty day lethal threshold concentration of Aroclor 1254 to steelhead trout lies between 10.15 and 20.40 $\mu\text{g/l}$. A threshold concentration of 17 $\mu\text{g/l}$ was determined by plotting a toxicity curve with five LC50 estimates made from the bioassay data according to the method

Figure 11. The results of a thirty day toxicity test in which young steelhead trout were exposed to five concentrations of Aroclor 1254. Mortality was incomplete in 10.15 $\mu\text{g}/\text{l}$ as indicated by the broken line (C). Fish growth was reduced at concentrations not acutely toxic.



MEDIAN SURVIVAL TIMES

<u>Line</u>	<u>PCB (ppb)</u>	<u>MST</u>	<u>C.I.</u>
A	39.40	293	256 - 335
B	20.40	400	337 - 475
C	10.15	—	—

Figure 11

Table 10. The length and weight of steelhead trout before and after exposure to various concentrations of Aroclor 1254.

PCB exposure conc. ($\mu\text{g/l}$)	Length (mm)		Weight (g)		Final n
	start ^b	finish	start ^a	finish	
0	47.43+4.47	58.27+7.22	1.03	1.89	37
2.55	49.03+4.49	56.75+5.19	1.03	1.78	40
4.75	47.34+4.39	54.50+6.32*	1.03	1.56	38
10.15	46.47+3.49	54.06+5.31*	1.03	1.42	34
20.40	47.82+3.80	56.25+4.66	1.03	1.73	8
39.40	47.40+4.37	53.30+6.12*	1.03	--	3

^aInitial n = 40 in all groups.

^bGroups are not significantly different, $p = .05$.

* Significantly different from control fish at $p = .05$.

recommended by Standard Methods (American Public Health Association et al., 1971). That the growth of the test fish was affected at levels below 16 µg/l PCB indicates the need for longer tests in evaluating the effects of PCB.

The acute toxicity of Aroclor 1254 to fish has been investigated by others. Mayer (1972) determined a 25 day LC50 of 27 µg/l Aroclor 1254 for rainbow trout (water temperature 17°C, alkalinity 159 mg/l) but stated that toxicity had not yet become independent of exposure time. Stalling (1970) estimated a median lethal time of 200 hours (8.3 days) for rainbow trout in a 50 µg/l solution of Aroclor 1254 at 20°C.

Two species of marine fish were exposed to 5 µg/l Aroclor 1254 by Hansen et al. (1971) for various periods of time up to 56 days. Pinfish (Lagodon rhomboides) showed 66% mortality after 14 days while 62% of spot (Leiostomus xanthurus) died in 45 days.

CONCLUSION

Young coho salmon and steelhead trout exposed to Aroclor 1254 concentrations above 15 $\mu\text{g}/\text{l}$ will be killed if the exposure period is long enough. Aroclor 1254 must therefore be classified as a compound very toxic to fish. It is unlikely however, that fish kills attributed to PCB poisoning in natural waters will be reported because 1) we know of no lakes or rivers presently suitable for aquatic life that have been found to have PCB levels above 15 $\mu\text{g}/\text{l}$ for sustained periods; 2) high PCB concentrations in natural waters are likely to be complexed with suspended organic and inorganic material in the water and thus possibly be unavailable for direct uptake by fish; 3) the behavior of fish, through either natural movement patterns or avoidance reactions (Sprague and Drury, 1969; Hansen, 1969) is such that lingering in polluted water for extended periods of time would be unlikely.

Our results show that 5 $\mu\text{g}/\text{l}$ Aroclor 1254 affected the growth of salmon and trout after less than 30 days exposure. Longer tests, if the experiences of other investigators with other toxicants are applicable to this case, will show that much lower levels of PCB are detrimental to fish growth and reproduction. Such tests are presently being conducted at

the United States National Water Quality Laboratory, Duluth, Minnesota.

Biologists attempting long term bioassays with chlorinated hydrocarbons like PCB are presented with difficult problems. For example, how does one go about determining whether the observed reductions in Lake Michigan coho salmon reproduction are related to PCB (or DDT) residues in the eggs? To run a chronic test, exposure concentrations would have to be 1 to 30 ng/l (pptr), levels hard to detect analytically. The fish food, if not natural, would have to be fortified with semi-"natural" levels of PCB residues. The exposure system would have to be large and the exposure period long--a minimum of one year and possibly three. Furthermore, a duplicate experiment would probably have to be conducted in which both PCB and DDT were incorporated into the water and food. What guarantee would there be that when the experiment was nearing completion that the residues in the fish eggs would approach the levels found in natural waters? Costs for such tests would be very high and probably not justifiable unless the results could definitely contribute to legal proceedings in matters related to PCBs.

PCB pollution, as with other types of contamination, is most easily remedied by prevention. Fortunately, measures can and have been taken with respect to the PCB problem. Monsanto, the sole producer of PCB in this country, has modified the formulation of its "Aroclor" series and placed

restrictions upon many of their uses. States have initiated monitoring programs to locate the point sources for PCB contamination. The United States Environmental Protection Agency (EPA) has suggested an interim maximum permissible concentration of 10 ng/l (pptr) PCBs in natural waters. If this standard is enforced, industrial hygiene will have to be upgraded. If the PCB levels in receiving waters near industrial centers can be limited to 10 ng/l, the concentrations in dilutent bodies of water such as Lake Michigan should fall to undetectable, and hopefully biologically safe, levels within several years.

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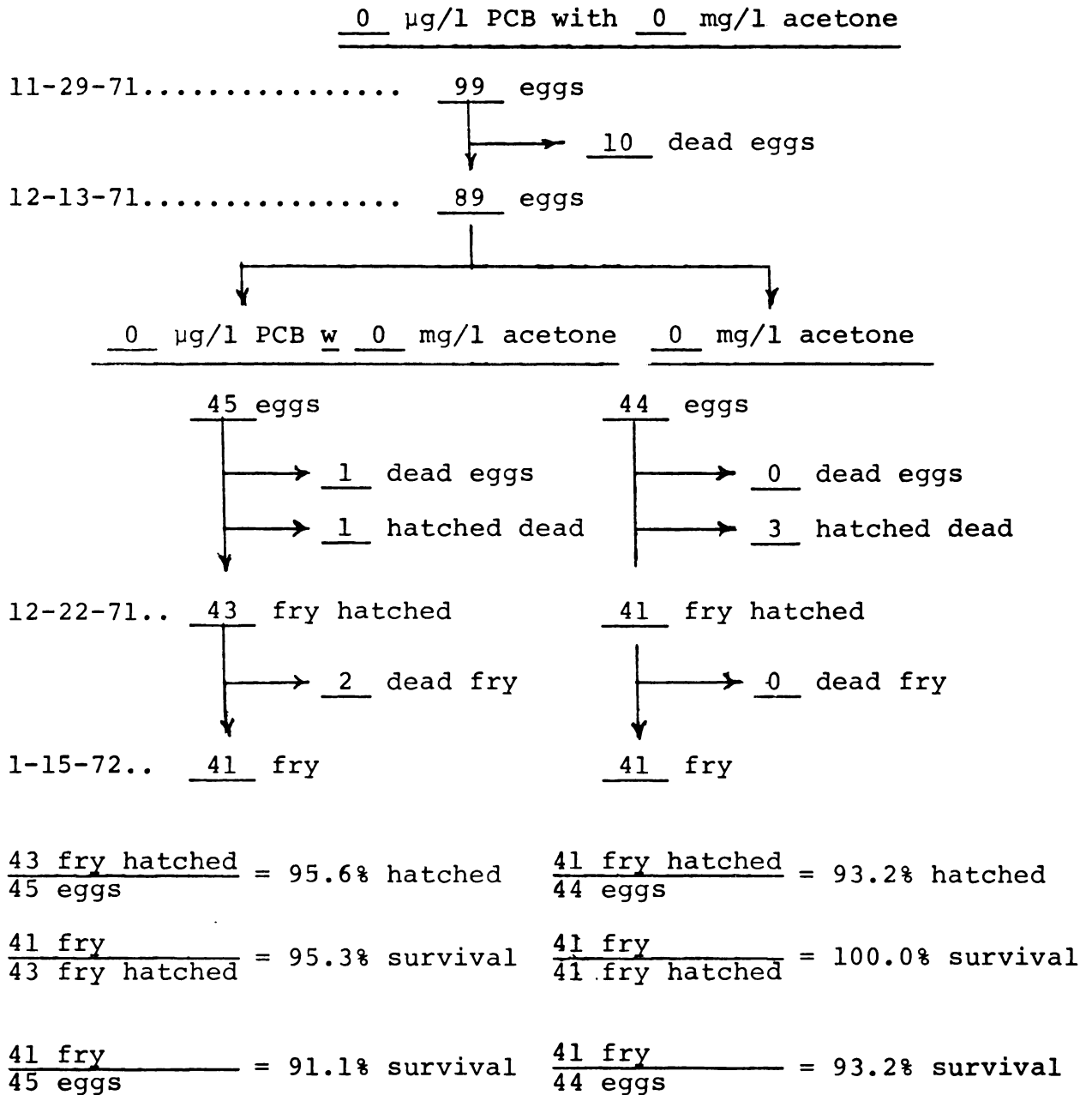
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APPENDIX

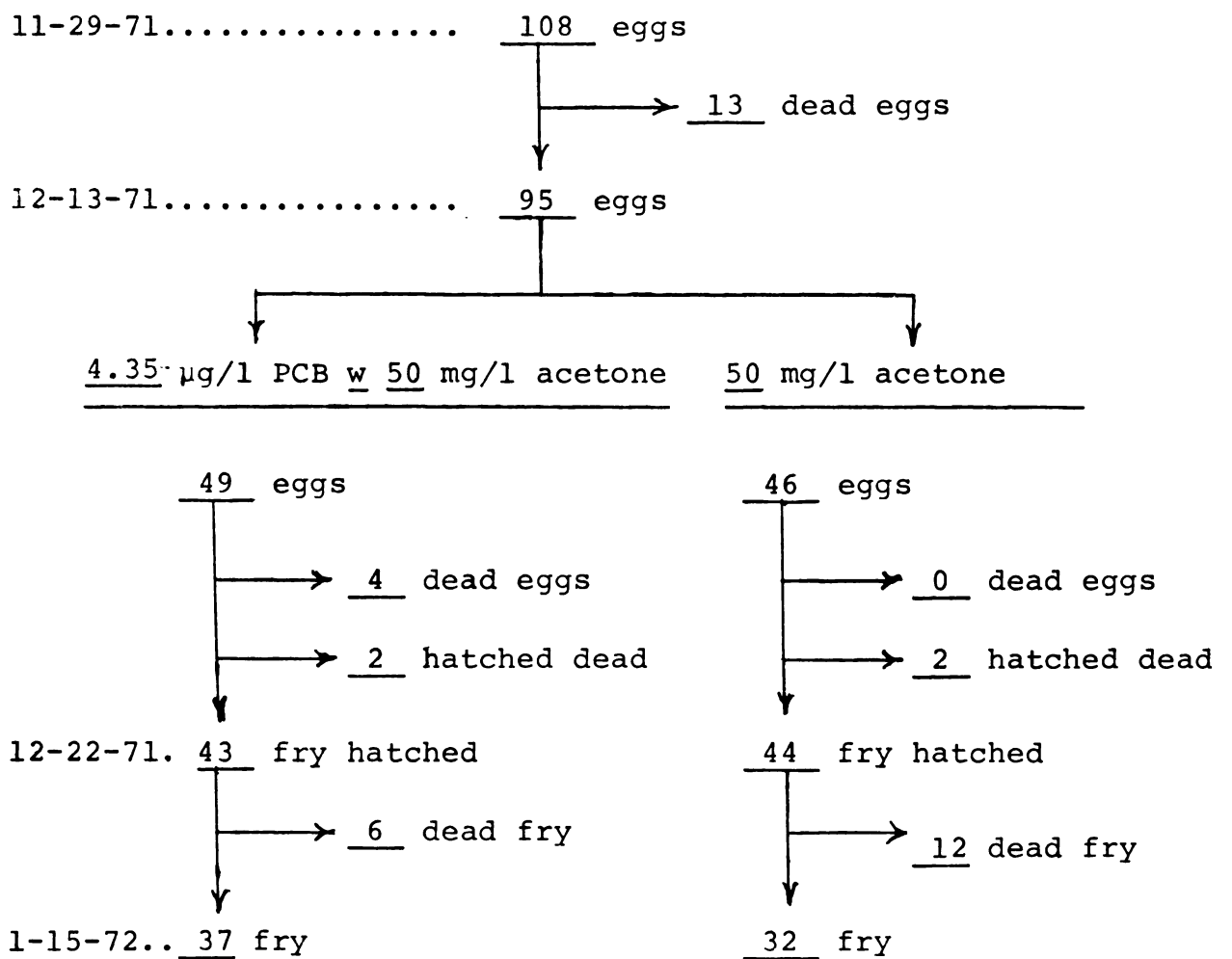
BREAKDOWN OF LOSSES OF EGG, SAC-FRY AND ALEVINS
EXPOSED TO VARIOUS CONCENTRATIONS
TO AROCLOR 1254

EGG-FRY EXPOSURE TEST



EGG-FRY EXPOSURE TEST

4.35 $\mu\text{g/l}$ PCB with 50 mg/l acetone



$$\frac{43 \text{ fry hatched}}{49 \text{ eggs}} = 87.8\% \text{ hatched}$$

$$\frac{44 \text{ fry hatched}}{46 \text{ eggs}} = 95.7\% \text{ hatched}$$

$$\frac{37 \text{ fry}}{43 \text{ fry hatched}} = 86.0\% \text{ survival}$$

$$\frac{32 \text{ fry}}{44 \text{ fry hatched}} = 72.7\% \text{ survival}$$

$$\frac{37 \text{ fry}}{49 \text{ eggs}} = 75.5\% \text{ survival}$$

$$\frac{32 \text{ fry}}{46 \text{ eggs}} = 69.6\% \text{ survival}$$

EGG-FRY EXPOSURE TEST

7.75 µg/l PCB with 100 mg/l acetone

11-29-71..... 110 eggs



→ 4 dead eggs

12-13-71..... 106 eggs



7.75 µg/l PCB w 100 mg/l acetone

56 eggs



→ 6 dead eggs

→ 6 hatched dead

12-22-71... 44 fry hatched



→ 8 dead fry

1-15-72.... 36 fry



100 mg/l acetone

56 eggs



→ 1 dead eggs

→ 4 hatched dead

45 fry hatched



→ 1 dead fry

44 fry

$$\frac{44 \text{ fry hatched}}{56 \text{ eggs}} = 78.6\% \text{ hatched}$$

$$\frac{45 \text{ fry hatched}}{50 \text{ eggs}} = 90.0\% \text{ hatched}$$

$$\frac{36 \text{ fry}}{44 \text{ fry hatched}} = 81.8\% \text{ survival}$$

$$\frac{44 \text{ fry}}{45 \text{ fry hatched}} = 97.8\% \text{ survival}$$

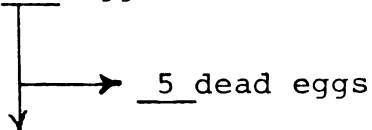
$$\frac{36 \text{ fry}}{44 \text{ eggs}} = 64.3\% \text{ survival}$$

$$\frac{44 \text{ fry}}{50 \text{ eggs}} = 88.0\% \text{ survival}$$

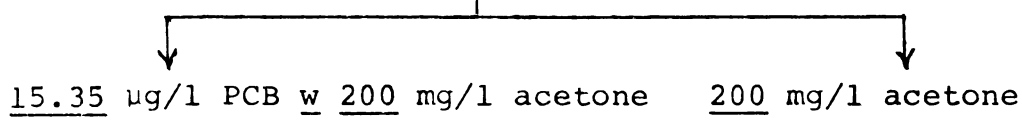
EGG-FRY EXPOSURE TEST

15.35 µg/l PCB with 200 mg/l acetone

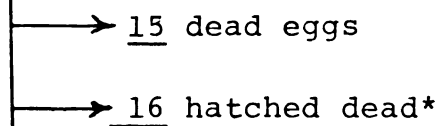
11-29-71..... 103 eggs



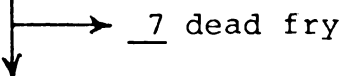
12-13-71..... 98 eggs



50 eggs

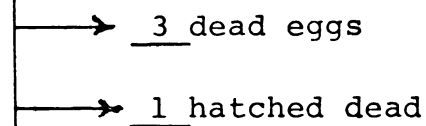


12-22-71.. 19 fry hatched

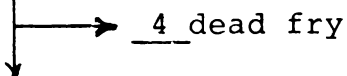


1-15-72... 12 fry

48 eggs



44 fry hatched



40 fry

$$\frac{19 \text{ fry hatched}}{50 \text{ eggs}} = 38.0\% \text{ hatched*}$$

$$\frac{44 \text{ fry hatched}}{48 \text{ eggs}} = 91.7\% \text{ hatched}$$

$$\frac{12 \text{ fry}}{19 \text{ fry hatched}} = 63.2\% \text{ survival}$$

$$\frac{40 \text{ fry}}{44 \text{ fry hatched}} = 90.9\% \text{ survival}$$

$$\frac{12 \text{ fry}}{50 \text{ eggs}} = 24.0\% \text{ survival}$$

$$\frac{40 \text{ fry}}{48 \text{ eggs}} = 83.3\% \text{ survival}$$

*Bacteria or fungi caused excessive egg mortality in these chickens.

EGG-FRY EXPOSURE TEST

25.90 µg/l PCB with 400 mg/l acetone

11-29-71..... 109 eggs

↓ → 7 dead eggs

102 eggs

25.90 µg/l PCB w 400 mg/l acetone

400 mg/l acetone

59 eggs

↓ → 1 dead eggs

↓ → 1 hatched dead

12-22-72.. 57 fry hatched

↓ → 53 dead fry

1-15-72... 4 fry

43 eggs

↓ → 1 dead eggs

↓ → 0 hatched dead

42 fry hatched

↓ → 10 dead fry

32 fry

$\frac{57 \text{ fry hatched}}{59 \text{ eggs}} = 96.6\% \text{ hatched}$

$\frac{42 \text{ fry hatched}}{43 \text{ eggs}} = 97.7 \text{ hatched}$

$\frac{4 \text{ fry}}{57 \text{ fry hatched}} = 7.0\% \text{ survival}$

$\frac{32 \text{ fry}}{42 \text{ fry hatched}} = 76.2\% \text{ survival}$

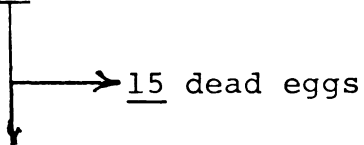
$\frac{4 \text{ fry}}{59 \text{ eggs}} = 6.8\% \text{ survival}$

$\frac{32 \text{ fry}}{43 \text{ eggs}} = 74.4\% \text{ survival}$

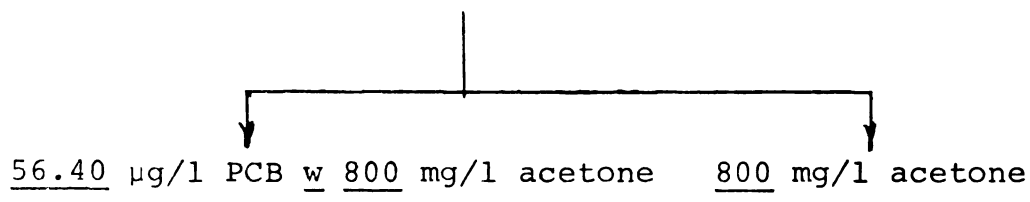
EGG-FRY EXPOSURE TEST

56.40 µg/l PCB with 800 mg/l acetone

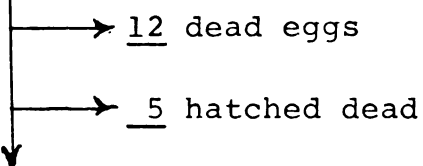
11-29-71..... 99 eggs



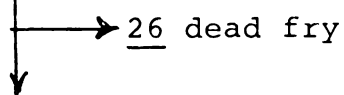
12-13-71..... 84 eggs



46 eggs

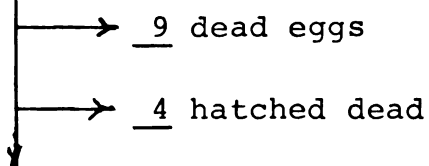


12-22-71.. 29 fry hatched

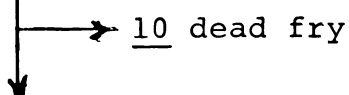


1-15-72... 3 fry

38 eggs



25 fry hatched



15 fry

$$\frac{29 \text{ fry hatched}}{46 \text{ eggs}} = 63.0\% \text{ hatched}$$

$$\frac{25 \text{ fry hatched}}{38 \text{ eggs}} = 65.8\% \text{ hatched}$$

$$\frac{3 \text{ fry}}{29 \text{ fry hatched}} = 10.3\% \text{ survival}$$

$$\frac{15 \text{ fry}}{25 \text{ fry hatched}} = 60.0\% \text{ survival}$$

$$\frac{3 \text{ fry}}{46 \text{ eggs}} = 6.5\% \text{ survival}$$

$$\frac{15 \text{ fry}}{38 \text{ eggs}} = 39.5\% \text{ survival}$$

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