December 1976

Ecological Research Series

# EFFECTS OF AROCLOR® 1254 ON BROOK TROUT, *Salvelinus fontinalis*


Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804

## RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/3-76-112
December 1976

# EFFECTS OF AROCLOR® 1254 ON BROOK TROUT, SALVELINUS FONTINALIS

by

Virginia M. Snarski
Frank A. Puglisi
Environmental Research Laboratory-Duluth
Duluth, Minnesota 55804

ENVIRONMENTAL RESEARCH LABORATORY-DULUTH
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MINNESOTA 55804

## DISCLAIMER

This report has been reviewed by the Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

# FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry---physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota develops methods, conducts laboratory and field studies, and extrapolates research findings

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man

This report describes the effects of a long-term exposure of brook trout to the polychlorinated biphenyl, Aroclor® 1254, at extremely low concentrations in the water (0.01 - 0.94 µg/l). Because of the persistence of these chlorinated hydrocarbons and their tendency to bioaccumulate, the measurement of tissue concentrations was an important part of the study.

Donald I. Mount, Ph.D.
Director
Environmental Research Laboratory
Duluth, Minnesota

## ABSTRACT

No adverse effects were observed on survival, growth, and reproduction of brook trout exposed for 71 weeks to 0.94 µg/l. and lower concentrations of the polychlorinated biphenyl Aroclor® 1254 ($P = 0.05$). Survival and growth to 90 days of alevin-juveniles from exposed parents were also unaffected ($P = 0.05$). Polychlorinated biphenyl concentrations in the brook trout were directly proportional to the water exposure concentration ($P = 0.05$). The PCB tissue concentrations appeared to have reached a steady state by the first sampling after 14 weeks of exposure. The PCB residues (wet-tissue basis) in chronically exposed fish were approximately 2 µg/g in the fillet and 9 µg/g in the "whole body" (entire fish minus one fillet and the gonads) at the highest water concentration, 0.94 µg/l. The higher residue in the whole body compared to the corresponding fillet was due to the higher fat content of the former.

CONTENTS

TABLES

ACKNOWLEDGMENTS

The authors thank Mr. C. Walbridge for collecting PCB water samples, for making routine water analyses, and for daily assistance; Mr. L.F. Mueller for assisting in PCB water analysis and constructing test equipment; and the Environmental Research Laboratory-Duluth's staff for technical assistance, advice, and manuscript review. We would also like to thank the Monsanto Company for kindly supplying the Aroclor® 1254 used in this experiment.

# SECTION 1

# INTRODUCTION

The objective of this study was to determine the effects of 1 µg/l. and lower water concentrations of the polychlorinated biphenyl (PCB) Aroclor® 1254* on the life cycle of the brook trout, Salvelinus fontinalis (Mitchill). Widespread PCB contamination of the environment has occurred (Jensen, 1966; Koeman et al., 1969; Duke et al., 1970, Veith, 1972; Giam et al., 1973; and others). Although laboratory studies have shown adverse effects on survival and reproduction and biological accumulation from low (µg/l.) PCB concentrations on other fish species (Hansen et al., 1971, 1973; Stalling and Mayer, 1972; Nebeker et al., 1974; Schimmel et al., 1974), no studies on the effects of long-term exposure to known concentrations of PCB's on salmonids could be found in the literature. Because of similarities in chemical properties and biological activity, demonstrated particularly in birds, between PCB's and DDT (Risebrough and Brodine, 1970), this experiment was designed to determine if sublethal PCB concentrations might have effects on salmonids similar to those observed with DDT (Burdick et al., 1964, 1972; Allison et al., 1964; Macek, 1968). The uptake of PCB residues from chronically exposed females and transfer to their ova as well as the survival of embryos and alevins at the yolk-sac absorption stage were of particular interest.

*Registered trademark of Monsanto Co., St. Louis, MO.

## SECTION 2

## CONCLUSIONS

No adverse effects were observed on survival and growth of first-generation brook trout during 71 weeks of exposure or on their progeny exposed for 90 days to Aroclor® 1254 concentrations of 0.01-0.94 µg/1. (P = 0.05).

Polychlorinated biphenyl concentrations in fillets and whole bodies (entire fish minus one fillet above the lateral line and gonads) of the brook trout reached an apparent steady state by the first sampling period after 14 weeks of exposure.

Exposure of brook trout to 0.01-0.94 µg/1. Aroclor$^{R}$ 1254 resulted in mean PCB residues from less than detectable (<0.2) to 2 µg/g in fillets and from 0.5 to 9 µg/g in whole bodies. The differences in PCB concentration between fillet and whole body samples at a given water concentration were directly related to their fat content.

Linear regression analyses showed the PCB concentration in the tissue to be directly proportional to the concentration in the water. Concentration factors in whole bodies of brook trout of 10,000-42,000 times the water concentration agree with the concentration factors of 20,000-70,000 for other species of fish observed by several other investigators.

## SECTION 3

## RECOMMENDATIONS

It is recommended that further research be conducted at higher PCB concentrations to determine if increased residues in parental fish cause effects on their offspring.

It is recommended that additional studies be conducted to determine factors that might affect PCB residue levels in different species of fish. Concentration factors observed in this study with brook trout ranged from 10,000 to 42,000, whereas those in fathead minnows were around 200,000 (Nebeker et al., 1974). The roles of such factors as feeding habits, water temperatures, and water chemistry and possibly other behavioral differences deserve attention.

## SECTION 4

## MATERIALS AND METHODS

### BIOASSAY

The long-term exposure to assess the effect of Aroclor® 1254 on survival, growth, and reproduction of brook trout was conducted according to the recommended procedures of the Environmental Research Laboratory-Duluth [APPENDIX], except as noted below.

Lake Superior water passed through an ultraviolet light sterilizer was used throughout the study. Mean water quality characteristics (± standard deviation) in the exposure tanks were: acidity, 6.0±2.0 mg/l.; alkalinity, 43.1±1.4 mg/l.; total hardness, 45.8±1.8 mg/l. (as $CaCO_3$); dissolved oxygen, 78±14 percent saturation; pH, 7.2 (mode). Aroclor® 1254 had no measurable effect on any of these parameters.

A proportional diluter (Mount and Brungs, 1967) delivered 4 l./cycle of each of five Aroclor® 1254 concentrations and a lake-water control. The flow rate was maintained at approximately 100 l. of each concentration every hour. A flow splitter (Benoit and Puglisi, 1973) was used to divide the flow between duplicate spawning tanks, A and B, and later among duplicate spawning tanks and duplicate incubation-growth tanks. Because the solubility of Aroclor® 1254 in water is low, acetone was used as a carrier in the stock solution. Acetone was also added to the control water so that it received the same concentration (0.004 ml/l.) as the high PCB concentration. An injector, designed to hold two 50-ml. glass syringes, delivered the PCB stock solution and acetone simultaneously to appropriate diluter chambers at each cycle.

Young-of-the-year brook trout obtained from Cedar Bend Hatchery in Scandia, Minnesota, were acclimated to the water supply and beginning test temperature for 2 months. Forty 10-month-old fish were then distributed by stratified random assignment to each duplicate spawning tank. In addition, a random sample of 40 fish was taken, and the fish were weighed, measured, and stored at -20° C for later determination of background PCB tissue residues. Mean weight and total length of these trout were 3.93 g and 7.3 cm, respectively.

The fish were fed PR-6 trout food† for 11 months until a formulation change necessitated changing to a gelatin-based purified diet (Castell et al., 1972) to minimize pesticide residues that might complicate interpretation of the PCB experiment. The progeny were fed EWOS Salmon Starter‡.

Total lengths and weights were recorded as the fish were sampled (see below). Analysis of variance of the logarithmic transformation of body weight was used to detect treatment difference. Survival data for each sampling period were subjected to analysis of variance and Dunnett's test when required.

During spawning two substrates (Benoit, 1974) were provided in each tank. Because viability was low in many spawnings at all exposure levels, eggs in addition to those recommended in the bioassay procedure [APPENDIX] were incubated in hatchability cups to provide sufficient larvae for observation of possible toxicant effects. Analyses of variance of eggs per female, percent viability, and percent hatchability (arc sin $\sqrt{\text{percentage}}$ transformation used for viability and hatchability data) were made.

Ninety-day survival and growth studies of the offspring were also conducted [APPENDIX]. Data were subjected to analysis of variance and Student's t-test (Steel and Torrie, 1960).

Water concentrations of PCB in the test tanks were measured on 5-day composite water samples by gas chromatography (Table 1). Each day the PCB's were extracted from 1-l. water samples onto polyurethane foam plugs (Gesser et al., 1971). The PCB residues were composited for 1 week and were then extracted from the foam plugs with aliquots of redistilled acetone and hexane. Before gas chromatographic measurement, a Florisil column clean-up was used to reduce background contaminants that co-extracted with the PCB's. Quantitation was based upon the height of the major Aroclor® 1254 peak compared to the same peak of the standard.

## RESIDUE SAMPLING AND ANALYSIS

To determine the uptake of PCB's from the water, fish were sampled after 14, 27, 36, 41.5, 48, 55.5, 60, and 71 weeks of exposure. Five fish were randomly selected from each tank during the first six samplings. The seventh sampling, at 60 weeks, consisted of the fish discarded after thinning each tank to two males and four females in preparation for spawning [APPENDIX]. After 71 weeks of exposure, 2 weeks after the last spawning, the remaining brook trout were sampled. A fillet from the left side of the body above the

---

†Manufactured by Glencoe Mills, Glencoe, Minn. 55336.

‡A product of EWOS of Sweden, sold in the United States by Astra Pharmaceutical Co., Worcester, Mass.

TABLE 1. AROCLOR® 1254 CONCENTRATIONS (µg/l.) IN DUPLICATE BROOK TROUT ADULT AND ALEVIN-JUVENILE TANKS MEASURED BY GAS CHROMATOGRAPHY (CORRECTED FOR RECOVERY)

| Nominal concentration | | Adult tanks | | | Alevin-juvenile tanks | | |
|---|---|---|---|---|---|---|---|
| | | N | Mean | Standard deviation | N | Mean | Standard deviation |
| Control | A | 35 | 0.00 ± | 0.01 | 6 | 0.00 ± | 0.00 |
| | B | 35 | 0.00 | 0.01 | 6 | 0.00 | 0.00 |
| 0.012 | A | 37 | 0.01 | 0.01 | 7 | 0.01 | 0.00 |
| | B | 37 | 0.01 | 0.02 | 8 | 0.01 | 0.01 |
| 0.036 | A | 39 | 0.03 | 0.01 | 8 | 0.03 | 0.02 |
| | B | 37 | 0.03 | 0.02 | 8 | 0.02 | 0.01 |
| 0.11 | A | 36 | 0.08 | 0.03 | 9 | 0.07 | 0.03 |
| | B | 36 | 0.08 | 0.03 | 8 | 0.08 | 0.03 |
| 0.33 | A | 39 | 0.24 | 0.04 | 7 | 0.28 | 0.11 |
| | B | 32 | 0.25 | 0.05 | 8 | 0.23 | 0.06 |
| 1.0 | A | 39 | 1.01 | 0.23 | 9 | 1.23 | 0.36 |
| | B | 33 | 0.86 | 0.29 | 9 | 1.07 | 0.27 |

lateral line, the gonads, and the remainder of the body were frozen for residue analysis. Samples from one duplicate at the three highest PCB concentrations (0.08, 0.24, and 0.94 µg/l.) at 48 weeks and all samples at 55.5 weeks were individually wrapped in acetone-rinsed aluminum foil for separate residue analysis to obtain information on biological variability in PCB accumulation. For each of the other samplings, fish were pooled to make one composite of each sample type from each tank. Composite samples of newly spawned eggs from each tank were also frozen for PCB analysis. A sufficient number of eggs (75-125) were composited to obtain a sample of approximately 5 g.

The frozen fish tissues and eggs were packed in dry ice and shipped to the Analytical-Biochemical Laboratories in Columbia, Missouri, for analysis. There the samples were homogenized while still frozen, and approximately 10-g aliquots were mixed with 30 g of anhydrous sodium sulfate. The mixture was placed in a chromatographic column and washed with approximately 250 ml of 15% ether and hexane. The fat content was determined gravimetrically on an aliquot of the extract. The remaining extract was cleaned-up by placing it on a 20-g Florisil column and diluting it with 150 ml of 15% ethyl ether in hexane. The eluant was concentrated and was then injected into a gas chromatograph.

The PCB's were quantitated by summing the peak heights of peak numbers 70, 84, 125, 145, and 174, relative to DDE, and comparing the sum to the Aroclor® 1254 standard. Concentrations were expressed in micrograms of PCB per gram of tissue, wet weight.

# SECTION 5

# RESULTS

## BIOASSAY

No significant difference (P = 0.05) in survival was noted at any Aroclor® 1254 concentration compared to the control during the first 60 weeks of exposure (Table 2). The increased mortality during the remaining 11 weeks of exposure was not believed to be due to the PCB. During this latter period a disease of undetermined etiology occurred producing lesions and other signs suggestive of furunculosis (Bullock et al., 1971). All but one of the fish that died within this period displayed these pathological signs. The fish in every tank were treated by incorporation of tetracycline hydrochloride (at approximately 7.5 g/100 kg body weight per day) into the food for 14 consecutive days.

No statistically significant difference in growth between any PCB concentration and the control was observed during any period of the exposure (P = 0.05). Growth data from the final sampling period only (after 71 weeks) are presented in Table 2.

Spawning occurred at all PCB concentrations and in the controls after approximately 14 months of exposure. No significant difference in total spawning or eggs per female could be detected between the PCB treatments and the controls (P = 0.05) (Table 2). Viability of eggs was highly variable at all PCB concentrations and in the controls. Eggs from many spawnings were entirely nonviable. The reason for this poor viability is not known; however, since it occurred in controls as well as in the toxicant-containing tanks, it could not be attributed to the Aroclor® 1254. Hatchability of eggs incubated reflected the erratic viability (Table 3). However, mean hatchabilities of viable eggs were 93-100% for the control and all Aroclor® 1254 concentrations tested. [Viability is defined as the formation of a neural keel after 11-12 days at 9° C (APPENDIX).]

No significant differences (P = 0.05) in survival and growth between controls and any PCB concentration tested were noted during any part of the 90-day alevin-juvenile exposure (Table 3).

## PCB TISSUE RESIDUES

Concentrations of PCB's in the unexposed brook trout sampled at the beginning of the experiment were less than detectable (<0.2 µg/g wet weight).

TABLE 2. SURVIVAL, GROWTH, AND REPRODUCTION OF BROOK TROUT EXPOSED TO AROCLOR® 1254 FOR 16 MONTHS

| Mean measured PCB concentration (µg/l.) | | Cumulative mortality (Weeks) 60 | 71 | Length (cm) and weight (g) at termination (71 weeks) Males cm | g | Females cm | g | Sex ratio in each tank[a] male/female | Total spawnings[d] | Mean eggs/female | Mean viable eggs/female | Viability (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control 0.00 | A[c] | 1 | 1 | 29.9[d] (2.2)[e] | 308.4 (60.1) | 26.5[d] (1.8) | 175.7 (21.9) | 2/4 | 10 | 641 | 449 | 70 |
| | B[c] | 0 | 0 | 4[f] | | 8 | | 2/4 | 15 | 720 | 160 | 22 |
| 0.01 | A | 1 | 5 | 30.1 (3.6) | 294.7 (92.1) | 27.9 (2.2) | 210.2 (43.7) | 1/2 | 7 | 749 | 137 | 18 |
| | B | 0 | 1 | 2 | | 7 | | 1/3 | 15 | 911 | 62 | 7 |
| 0.03 | A | 0 | 2 | 27.1 (3.1) | 211.4 (64.2) | 28.4 (1.0) | 237.8 (32.0) | 1/3 | 14 | 870 | 324 | 37 |
| | B | 0 | 0 | 4 | | 6 | | 3/2 | 17 | 1,554 | 363 | 23 |
| 0.08 | A | 1 | 2 | 30.3 (0.1) | 294.0 (9.2) | 26.8 (2.0) | 185.1 (54.9) | 1/4 | 9 | 255 | 10 | 4 |
| | B | 1 | 2 | 2 | | 8 | | 1/4 | 12 | 579 | 124 | 21 |
| 0.24 | A | 1 | 2 | 27.3 (2.4) | 249.0 (49.5) | 26.5 (1.7) | 187.3 (37.5) | 2/4 | 13 | 630 | 126 | 20 |
| | B | 1 | 4 | 3 | | 5 | | 1/3 | 6 | 615 | 500 | 81 |
| 0.94 | A | 0 | 1 | 27.4 (2.0) | 231.3 (36.6) | 26.3 (2.3) | 179.1 (35.4) | 2/4 | 9 | 489 | 231 | 47 |
| | B | 0 | 1 | 4 | | 6 | | 2/2 | 5 | 852 | 476 | 56 |

[a] Represents fish present during spawning period that contributed to spawning data.

[b] A spawning is defined as any egg deposition of 50 or more eggs.

[c] A and B are duplicate spawning tanks.

[d] Data from duplicate tanks combined.

[e] Standard deviation in parentheses.

[f] Number of fish.

TABLE 3. HATCHABILITY, SURVIVAL, AND GROWTH OF BROOK TROUT ALEVIN-JUVENILES FROM PARENTS EXPOSED TO AROCLOR® 1254 FOR 14 MONTHS BEFORE SPAWNING

| Mean measured PCB concentration (µg/l.) | | Mean percent hatch | Alevin-juvenile survival (%) | | Alevin-juvenile 90-day growth | |
|---|---|---|---|---|---|---|
| | | | Hatch- | | Mean | Mean |
| | | | 30 days | 30-90 days | length (mm) | weight (g) |
| Control 0.00 | A[a] | 57 [9][b] | 100 (175)[c] | 94 (50)[c] | 41 | 0.69 |
| | B[a] | 5 [15] | -[d] | 68[e] (25) | 34 | 0.40 |
| 0.01 | A | 14 [7] | 100 (49) | 100 (46) | 43 | 0.87 |
| | B | 5 [10] | 100 (50) | 88 (50) | 42 | 0.72 |
| 0.03 | A | 19 [12] | 96 (71) | 84 (49) | 39 | 0.68 |
| | B | 13 [15] | 99 (126) | 84 (50) | 34 | 0.44 |
| 0.08 | A | 8 [9] | 100 (29) | 88 (25) | 38 | 0.57 |
| | B | 8 [11] | 100 (50) | 78 (50) | 44 | 0.99 |
| 0.24 | A | 15 [10] | 99 (74) | 65 (46) | 34 | 0.47 |
| | B | 66 [7] | 100 (100) | 60 (50) | 35 | 0.54 |
| 0.94 | A | 45 [8] | 100 (156) | 84 (50) | 33 | 0.41 |
| | B | 75 [5] | 98 (148) | 94 (47) | 33 | 0.42 |

[a] Duplicate chambers.

[b] [ ] Number of hatch cups incubated.

[c] ( ) Initial number of alevins.

[d] All fish killed before 30 days by temperature increase in this tank.

[e] One group of 25 fry transferred from duplicate after the resident fish died.

The results of the PCB residue analyses of fillet and "whole body" (entire fish minus one fillet and the gonads) after various lengths of exposure to Aroclor® 1254 are presented in Tables 4 and 5, respectively. Because no biological effects were observed on embryos and alevins, no gonads were analyzed. Both tables suggest that the brook trout had reached an apparent steady state by the first sampling after 14 weeks of exposure. Linear regression analysis for each sampling period showed the PCB concentration in the tissue to be directly proportional to the PCB concentration in the water. (Coefficients of correlation ranged from 0.970 to 0.999, all statistically significant at $P = 0.05$ with only 60- and 71-week R-values not significant also at $P = 0.01$.) The PCB concentrations in the fillet were below detectability (<0.2 µg/g) at water concentrations of 0.03 µg/l. and less. Exposure to 0.94 µg/l., the highest water concentrations tested, resulted in PCB residues of approximately 2 µg/g in the fillet and 9 µg/g in the whole body.

Limited information on PCB residues in newly spawned eggs was obtained because of the availability of only a small number of samples and variability within these samples. Control eggs and those from 0.01 µg/l. had less than detectable amounts (<0.1 µg/g, N = 2 and 1, respectively). Eggs from brook trout exposed to 0.03 and 0.08 µg/l. contained PCB's at the lower detection limit (0.1 µg/g, N = 3 and 2, respectively). Mean residues of 1.8 and 1.7 µg/g were detected in eggs from the 0.24 and 0.94 µg/l. concentrations (standard deviations = 1.3 and 0.1, N = 3 and 2, respectively). Percentages of fat ranged from 0.4 to 7.3 with a mean of 1.8% (N = 13). No relationship was evident between fat content and magnitude of PCB residue in the eggs.

TABLE 4. CONCENTRATION OF PCB (µg/g wet weight) AND FAT CONTENT (%) OF FILLETS FROM BROOK TROUT EXPOSED TO AROCLOR® 1254 FOR VARIOUS TIME PERIODS. VALUES REPRESENT AN ANALYSIS ON A COMPOSITE SAMPLE OF FIVE FISH UNLESS OTHERWISE NOTED.

| PCB concentration (µg/l.) | | Length of exposure: 14 weeks PCB µg/g | 14 weeks Percent fat | 27 weeks PCB µg/g | 27 weeks Percent fat | 36 weeks PCB µg/g | 36 weeks Percent fat | 41.5 weeks PCB µg/g | 41.5 weeks Percent fat | 48 weeks PCB µg/g | 48 weeks Percent fat | 55.5 weeks PCB µg/g | 55.5 weeks Percent fat | 60 weeks PCB µg/g | 60 weeks Percent fat | 71 weeks PCB µg/g | 71 weeks Percent fat |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control 0.00 | A | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | B | <0.2 | 0.9 | <0.2 | 1.4 | <0.2 | 1.4 | <0.2 | 2.5 | <0.2 | 1.2 | <0.2[a] | 1.3 | - | - | - | - |
| 0.01 | A | <0.2 | 1.4 | <0.2 | 1.3 | <0.2 | 0.9 | <0.2 | 1.6 | <0.2 | 0.2 | <0.2[a] | 0.9 | - | - | - | - |
| | B | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 0.03 | A | <0.2 | 1.5 | <0.2 | 1.5 | <0.2 | 1.2 | <0.2 | 2.3 | <0.2 | 0.3 | 0.1[a] (50)[b] | 1.3 (24) | - | - | - | - |
| | B | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 0.08 | A | - | - | - | - | - | - | - | - | 0.1-0.2[a] | 0.4 | - | - | - | - | - | - |
| | B | <0.2 | 0.9 | 0.2 | 1.4 | 0.2 | 0.9 | 0.2 | 1.7 | 0.2 | 0.9 | 0.2[a] (109) | 1.4 (61) | 0.3[e] n=4 | 1.6 | 0.2 | 0.5 |
| 0.24 | A | 0.4 | 1.0 | 0.4 | 1.0 | 0.4 | 1.3 | 0.6 | 1.2 | 0.4 | 0.5 | 0.9[a] (41) | 1.5 (58) | 0.8 | 1.5 | 1.0 | 0.6 |
| | B | - | - | - | - | - | - | - | - | 0.4[a] (71) | 0.4 (61) | 1.0[a] (26) | 1.9 (28) | 0.9[c] n=3 | 1.3 | 0.9[c] n=3 | 0.8 |
| 0.94 | A | - | - | - | - | - | - | 1.6 | 1.9 | 1.1 | 0.5 | 2.4[a] (46) | 1.1 (29) | 2.9 n=4 | 1.6 | 1.8 | 0.5 |
| | B | 1.2 | 0.9 | 0.9 | 1.0 | 1.5 | 1.6 | - | - | 1.5[a] (38) | 0.7 (99) | 2.1[d] (42) | 1.5 (50) | 1.9[c] n=4 | 1.6 | 2.1 | 1.0 |

[a] Mean of individual analyses of five fish.

[b] ( ) = relative standard deviation. The RSD is the standard deviation expressed as a percentage of the mean.

[c] Analysis of composite sample from N fish.

[d] Mean of individual analyses of four fish; one omitted because of suspected mix-up in chromatograms.

TABLE 5. CONCENTRATION OF PCB (µg/g wet weight) AND FAT CONTENT (%) OF WHOLE BODY (ENTIRE FISH MINUS ONE FILLET AND GONADS) OF BROOK TROUT EXPOSED TO AROCLOR® 1254 FOR VARIOUS TIME PERIODS. VALUES REPRESENT AN ANALYSIS OF A COMPOSITE SAMPLE OF FIVE FISH UNLESS OTHERWISE NOTED.

| PCB concentration (µg/l.) | | Length of exposure: 14 weeks PCB µg/g | 14 weeks Percent fat | 27 weeks PCB µg/g | 27 weeks Percent fat | 36 weeks PCB µg/g | 36 weeks Percent fat | 41.5 weeks PCB µg/g | 41.5 weeks Percent fat | 48 weeks PCB µg/g | 48 weeks Percent fat | 55.5 weeks PCB µg/g | 55.5 weeks Percent fat | 60 weeks PCB µg/g | 60 weeks Percent fat | 71 weeks PCB µg/g | 71 weeks Percent fat |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control 0.00 | A | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | B | <0.2 | 5.7 | 0.4 | 5.4 | 0.4 | 8.6 | 0.3 | 6.9 | 0.5 | 8.0 | <0.2[a] | 7.0 | - | - | - | - |
| 0.01 | A | 0.4 | 6.8 | 0.5 | 6.8 | 0.6 | 4.1 | 0.4 | 7.0 | 0.6 | 6.4 | 0.5[a] (40) | 5.2 (41) | - | - | - | - |
| | B | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 0.03 | A | 0.6 | 6.9 | 0.6 | 5.4 | 0.6 | 9.3 | 0.4 | 6.3 | 0.8 | 8.0 | 0.8[b] (16) | 6.0 (48) | - | - | - | - |
| | B | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 0.08 | A | - | - | - | - | - | - | - | - | 1.1[a] (10)[c] | 6.0 (42) | - | - | - | - | - | - |
| | B | 1.3 | 7.5 | 1.3 | 7.9 | 0.8 | 5.1 | 0.9 | 6.1 | 1.3 | 7.9 | 1.7[a] (30) | 7.2 (15) | 0.8[d] n=4 | 4.6 | 0.9 | 2.3 |
| 0.24 | A | 2.9 | 7.7 | 2.0 | 4.1 | 2.8 | 8.4 | 3.3 | 10.4 | 2.5 | 5.6 | 4.9[a] (20) | 6.3 (35) | 2.1 | 3.6 | 4.4 | 5.4 |
| | B | - | - | - | - | - | - | - | - | 3.5[a] (20) | 7.2 (20) | 3.7[a] (28) | 6.5 (30) | 3.4[d] n=3 | 5.6 | 3.5[d] n=3 | 2.1 |
| 0.94 | A | - | - | - | - | - | - | 7.9 | 7.8 | 10.7 | 8.4 | 10.9[a] (26) | 6.6 (18) | 7.2[d] n=4 | 3.2 | 9.4 | 2.4 |
| | B | 6.2 | 5.3 | 8.9 | 7.4 | 8.0 | 8.2 | - | - | 10.8[a] (21) | 6.0 (11) | 12.3[a] (42) | 6.3 (31) | 6.8[d] n=4 | 6.4 | 7.2 | 2.4 |

[a] Mean of individual analyses of five fish.

[b] Mean of individual analyses of four fish; one omitted because of suspected mix-up in chromatograms.

[c] ( ) = relative standard deviation. The RSD is the standard deviation expressed as a percentage of the mean.

[d] Analysis of composite sample of N fish.

## SECTION 6

## DISCUSSION

In this experiment no adverse effects were noted on survival and growth of brook trout or their progeny chronically exposed to 0.01-0.94 µg/1. Aroclor® 1254. Jensen et al. (1970) observed mortalities of 16-100% in field-collected Atlantic salmon (Salmo salar) embryos containing PCB residues of 0.4-1.9 µg/g wet weight (7.7-34 µg/g of fat). Stalling and Mayer (1972) subjected the data of Jensen et al. (1970) to a regression analysis and demonstrated a statistically significant (P = 0.01) direct correlation between PCB residue and mortality of the embryos. Though some PCB egg residues in this study (1.8 and 1.7 µg/g from 0.24 and 0.94 µg/1. water concentrations, respectively) were comparable to those reported by Jensen et al. (1970), this experiment provided no evidence that PCB's transferred from parents cause any adverse effects on brook trout embryos. Also, in this experiment no unusual mortality occurred at yolk-sac absorption, which took place about 1 month post-hatch, as was observed in other salmonid alevins from DDT-fed parents (Burdick et al., 1964, 1972).

Concentration factors of 10,000-42,000 times the water exposure in whole bodies of brook trout were comparable to values of 20,000-71,000 for Aroclor® 1254 reported for bluegills (Lepomis macrochirus) and three species of estuarine fish by Stalling and Huckins (unpublished data) and Hansen et al. (1971, 1973). These residue concentration factors are considerably lower than values of around 200,000 reported by Nebeker et al. (1974) for fathead minnows (Pimephales promelas). The reasons for this discrepancy are not known; however, factors such as water temperatures and differences in fat content or feeding habits of the species may be partly responsible.

This study has shown, as others have (Reinert, 1970; Hamelink et al., 1971; Reinert and Bergman, 1974), that the fat content in the tissue plays an important role in determining the concentration of an organochlorine compound in various tissues from the same water concentration. The PCB concentrations in the brook trout fillets (Table 4) and whole bodies (Table 5) ranged from 2,000 to 3,000 and from 10,000 to 42,000, respectively, times higher than those in the water. Plotting the residue data as micrograms of PCB per gram of fat (Figure 1) reduces the differences in concentrations of PCB's between whole body and the corresponding fillet shown in Tables 4 and 5. As with the wet weight PCB concentrations, concentrations of PCB in fat were directly proportional to the water concentration (P = 0.01).

The apparent increase in the PCB content of the brook trout between 60 and 71 weeks (Figure 1) can be explained by a decrease in the fat content and

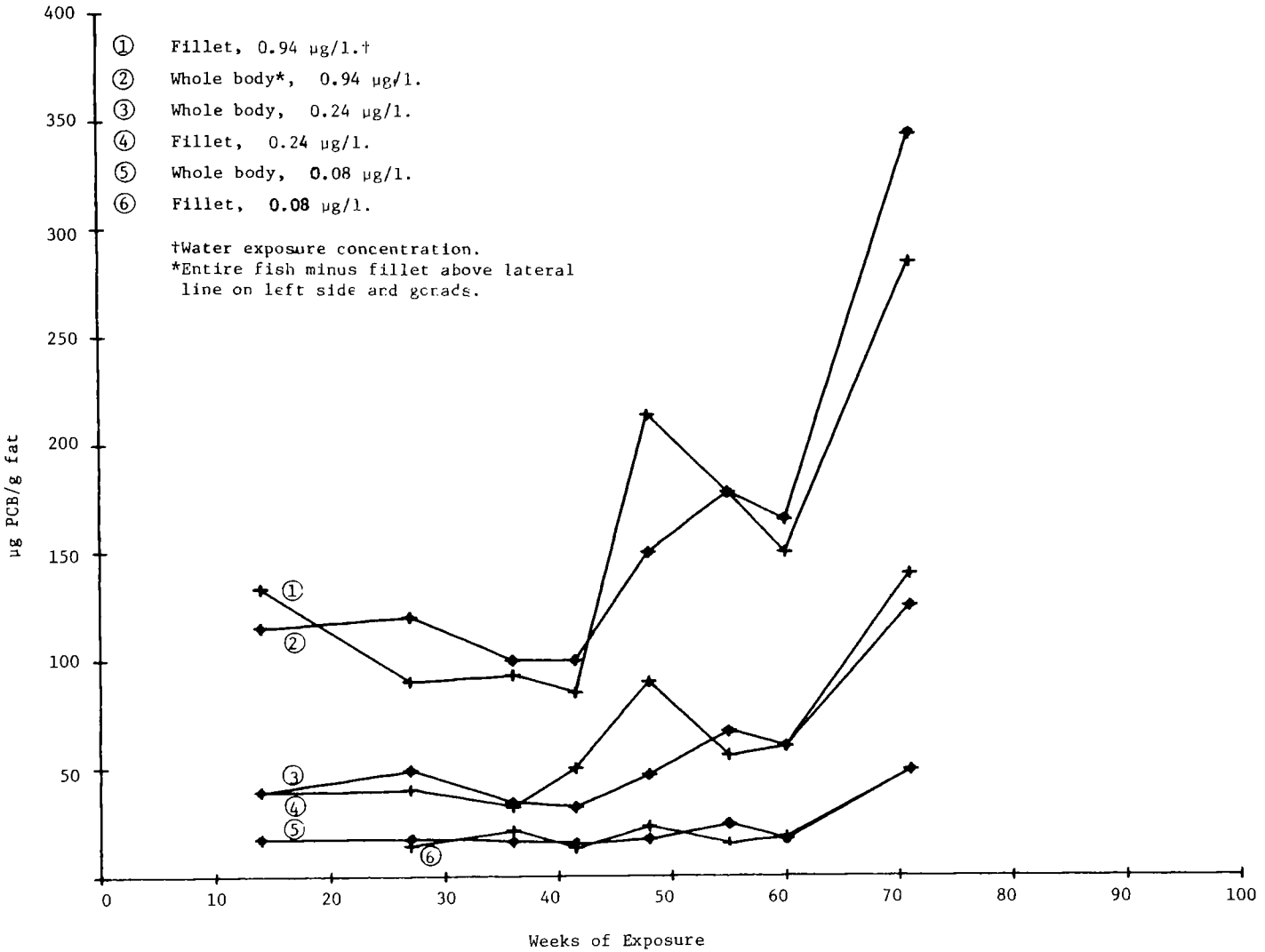


Figure 1. Concentration of PCB in fat brook trout tissues after various periods of exposure.

a further concentration of the PCB residues in the remaining fat. The percentage fat values at 71 weeks of both fillets and whole bodies are approximately one-half to one-third their previous values (Tables 4 and 5). During this period of exposure when the fish were spawning, increased activity, physiological stress, and a decreased feeding rate resulted in utilization of body-fat reserves. The hazards of otherwise sublethal PCB residues that might result from periods of prolonged mobilization to the point of complete depletion of fat reserves are not known. With another organochlorine compound, DDT, Grant and Schoettger (1972) reported high mortality of DDT-exposed rainbow trout (Salmo gairdneri) during prolonged exercise and fasting.

Bioconcentration of PCB's is important, not only because of the potential hazard of the residue to the fish, but also because of its economic and human-health implications. The U.S. Food and Drug Administration has set an interim tolerance level for PCB's in food of 5 µg/g, above which food is banned from the interstate market (U.S. Food and Drug Administration, 1974). In this experiment, PCB water concentrations of 0.24 and 0.94 µg/l. resulted in residues in whole bodies of brook trout approaching or exceeding the 5 µg/g action level. Because of the approximately five-fold lower fat content, fillets at none of the water concentrations tested exceeded the U.S. Food and Drug Administration's interim tolerance level. Since the U.S. Food and Drug Administration defines edible portion as an eviscerated, beheaded fish (U.S. Food and Drug Administration, 1969), our values for neither whole body nor fillet exactly represent their defined edible portion PCB concentrations.

## REFERENCES


Allison, D. T., B. J. Kallman, O. B. Cope, and C. Van Valin. 1964. Some chronic effects of DDT on cutthroat trout. U.S. Dept. Int., Bureau of Sport Fisheries and Wildlife Research Rept. 64. 30 p.

Benoit, D. A. 1974. Artificial laboratory spawning substrate for brook trout (Salvelinus fontinalis Mitchill). Trans. Am. Fish. Soc. 103:144-145.

Benoit, D. A., and F. A. Puglisi. 1973. A simplified flow-splitting chamber and siphon for proportional diluters. Water Res. 7:1915-1916.

Bullock, G. L., D. A. Conroy, and S. F. Snieszko. 1971. Diseases of fishes: Book 2A. Bacterial diseases of fish. T. F. H. Publications, Inc., N.J. 151 p.

Burdick, G. E., H. J. Dean, E. J. Harris, J. Skea, R. Karcher, and C. Frisa. 1972. Effect of rate and duration of feeding DDT on the reproduction of salmonid fishes reared and held under controlled conditions. N.Y. Fish & Game J. 19:97-115.

Burdick, G. E., E. J. Harris, H. J. Dean, T. M. Walker, J. Skea, and D. Colby. 1964. The accumulation of DDT in lake trout and the effect on reproduction. Trans. Am. Fish. Soc. 93:127-136.

Castell, J. D., R. O. Sinnhuber, J. H. Wales, and D. J. Lee. 1972. Essential fatty acids in the diet of rainbow trout (Salmo gairdneri): Growth, feed conversion, and some gross deficiency symptoms. J. Nutr. 102:77-86.

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. Environ. Contam. Toxicol. 5:171-180.

Gesser, H. D., A. Chow, F. C. Davis, J. F. Uthe, and J. Reinke. 1971. The extraction and recovery of polychlorinated biphenyls (PCB) using porous polyurethane foam. Anal. Lett. 4:883-886.

Giam, C. S., M. K. Wong, A. R. Hanks, W. M. Sackett, and R. L. Richardson. 1973. Chlorinated hydrocarbons in plankton from the Gulf of Mexico and Northern Caribbean. Bull. Environ. Contam. Toxicol. 9:376-382.

Grant, B. F., and R. A. Schoettger. 1972. The impact of organochlorine contaminants on physiologic functions in fish. Proc. Tech. Sess. 18th Ann. Meeting, Inst. Environ. Sci. pp. 245-250.

Hamelink, J. L., R. C. Waybrant, and R. C. Ball. 1971. A proposal: Exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. Trans. Am. Fish. Soc. 100:207-214.

Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr., and P. D. Wilson. 1971. Chronic toxicity, uptake, and retention of Aroclor® 1254 in two estuarine fishes. Bull. Environ. Contam. Toxicol. 6:113-119.

Hansen, D. J., S. C. Schimmel, and J. Forester. 1973. Aroclor® 1254 in eggs of sheepshead minnows: Effect on fertilization success and survival of embryos and fry. Proc. 27th. Ann. Conf. Southeastern Assoc. Game Fish Comm., 420-423.

Jensen, S. 1966. Report on a new chemical hazard. New Scientist and Sci. J. (Great Britain) 32:612.

Jensen, S., N. Johansson, and M. Olsson. 1970. PCB-indications of effects on salmon. PCB Conference, Stockholm, September 29, 1970. Swedish Salmon Research Institute Report LF1 MEDD 7/1970.

Koeman, J. H., M. C. Ten Noever De Braw, and R. H. DeVos. 1969. Chlorinated biphenyls in fish, mussels, and birds from the River Rhine and the Netherlands coastal area. Nature (London) 221:1126-1128.

Macek, K. J. 1968. Reproduction in brook trout (Salvelinus fontinalis) fed sublethal concentrations of DDT. J. Fish. Res. Board Can. 25:1787-1796.

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

Nebeker, A. V., F. A. Puglisi, and D. L. DeFoe. 1974. Effect of polychlorinated biphenyl compounds on survival and reproduction of the fathead minnow and flagfish. Trans. Am. Fish. Soc. 103:562-568.

Reinert, R. E. 1970. Pesticide concentrations in Great Lakes fishes. Pesticides Monitoring J. 3:233-240.

Reinert, R. E., and H. L. Bergman. 1974. Residues of DDT in lake trout (Salvelinus namacush) and coho salmon (Oncorhynchus kisutch) from the Great Lakes. J. Fish. Res. Board Can. 31:191-199.

Risebrough, R. W., and V. Brodine. 1970. More letters in the wind. Environment 12:16-27.

Schimmel, S. C., D. J. Hansen, and J. Forester. 1974. Effects of Aroclor® 1254 on laboratory-reared embryos and fry of sheepshead minnows (Cyrinodon variegatus). Trans. Am. Fish. Soc. 103:582-586.

Stalling, D. L., and J. N. Huckins. Unpublished from the U.S. Bureau of Sport Fisheries and Wildlife, Fish Pesticide Laboratory, Columbia, MO. 65201.

Stalling, D. L., and F. L. Mayer, Jr. 1972. Toxicities of PCB's to fish and environmental residues. Environ. Health Persp. April: 159-164.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics with special reference to biological sciences. McGraw-Hill, New York. 481 p.

U.S. Food and Drug Administration. 1969. Pesticide analytical manual, Vol. 1, Section 141.12c, July 1, 1969.

U.S. Food and Drug Administration. 1974. Interim tolerance for PCB in fish. Federal Register 39(6): part 2.

Veith, G. D. 1972. Recent fluctuations of chlorobiphenyls (PCB's) in the Green Bay, Wisconsin, region. Environ. Health Persp. April: 51-54.

# APPENDIX

## RECOMMENDED BIOASSAY PROCEDURE FOR

## BROOK TROUT SALVELINUS FONTINALES (MITCHILL) PARTIAL CHRONIC TESTS

## RECOMMENDED BIOASSAY PROCEDURES

### Preface

Recommended Bioassay Procedures are established by the approval of both the Committee on Aquatic Bioassays and the Director of the National Water Quality Laboratory. The main reasons for establishing them are: (1) to permit direct comparison of test results, (2) to encourage the use of the best procedures available, and (3) to encourage uniformity. These procedures should be used by National Water Quality Laboratory personnel whenever possible, unless there is a good reason for using some other procedure.

Recommended Bioassay Procedures consider the basic elements that are believed to be important in obtaining reliable and reproducible results in laboratory bioassays. An attempt has been made to adopt the best acceptable procedures based on current evidence and opinion, although it is recognized that alternative procedures may be adequate. Improvements in the procedures are being considered and tested, and revisions will be made when necessary. Comments and suggestions are encouraged.

Director, National Water Quality Lab (NWQL)

Committee on Aquatic Bioassays, NWQL

Recommended Bioassay Procedure for

Brook Trout Salvelinus fontinales (Mitchill) Partial Chronic Tests

April, 1971

(Revised January, 1972)

A. Physical system

1. Diluter: Proportional diluters (Mount and Brungs, 1967) should be employed for all long-term exposures. Check the operation of the diluter daily, either directly or through the measurement of toxicant concentrations. A minimum of five toxicant concentrations and one control should be used for each test with a dilution factor of not less than 0.30. An automatically triggered emergency aeration and alarm system must be installed to alert staff in case of diluter, temperature control or water supply failure.

2. Toxicant mixing: A container to promote mixing of toxicant bearing and w-cell water should be used between diluter and tanks for each concentration. Separate delivery tubes should run from this container to each duplicate tank. Check to see that the same amount of water goes to duplicate tanks and that the toxicant concentration is the same in both.

3. Tank: Each duplicate spawning tank (preferably stainless steel) should measure 1.3 X 3 X 1 ft. wide with a water depth of 1 foot and alevin-juvenile growth chambers (glass or stainless steel with glass bottom) 7 X 15 X 5 in. wide with a water depth of 5 inches. Growth chambers can be supplied test water by either separate delivery tubes from the mixing cells described in Step 2 above or from test water delivered from the mixing cell to each duplicate spawning tank. In the second choice, test water must always flow through growth chambers before entering the spawning tank. Each growth chamber should be designed so that the test water can be drained down to 1 inch and the chamber transferred over a fluorescent light box for photographing the fish (see B.10).

4. Flow rate: Flow rates for each duplicate spawning tank and growth chamber should be 6-10 tank volumes/24 hr.

5. Aeration: Brook trout tanks and growth chambers must be aerated with oil free air unless there are no flow limitations and 60% of saturation can be maintained. Total dissolved oxygen levels should never be allowed to drop below 60% of saturation.

6. Cleaning: All tanks and chambers must be siphoned daily and brushed at least once per week. When spawning commences, gravel baskets must be removed and cleaned daily.

7. Spawning substrates: Use two spawning substrates per duplicate made of plastic or stainless steel which measure at least 6 X 10 X 12 in. with 2 inches of .25 to .50 inch stream gravel covering the bottom and 20 mesh stainless steel or nylon screen attached to the ends for circulation of water.

8. Egg cup: Egg incubation cups are made from 4-oz. 2-inch OD round glass jars with the bottoms cut off and replaced with stainless steel or nylon screen (40 meshes per inch). Cups are oscillated in the test water by means of a rocker arm apparatus driven by a 2 r.p.m. electric motor (Mount, 1968).

9. Light: The lights used should simulate sunlight as nearly as possible. A combination of Duro-Test (Optima FS)[1,2] and wide spectrum Gro-lux[3] fluorescent tubes has proved satisfactory at the NWQL.

10. Photoperiod: The photoperiods to be used (Appendix A) simulate the dawn to dusk times of Evansville, Indiana. Evansville dates must correspond to actual dates in order to avoid putting natural reproductive cycles out of phase. Adjustments in photoperiod are to be made on the first and fifteenth of every Evansville test month. The table is arranged so that adjustments need be made only in the dusk times. The dawn and dusk times listed in the table (Evansville test time) need not correspond to the actual test times where the test is being conducted. To illustrate this point, a test started on March first would require the use of the photoperiod for Evansville test date March first, and the lights could go on any time on that day just so long as they remained on for twelve hours and fifteen minutes. Fifteen days later the photoperiod would be changed to thirteen hours. Gradual changes in light intensity at dawn and dusk (Drummond and Dawson, 1970), may be included within the photoperiods shown, and should not last for more than 1/2 hour from full on to full off and vice versa.

11. Temperature: Utilize the attached temperature regime (see Appendix B). Temperatures should not deviate instantaneously from the specified test temperature by more than 2° C and should not remain outside the specified temperature ±1° C for more than 48 hours at a time.

12. Disturbance: Spawning tanks and growth chambers must be covered with a screen to confine the fish and concealed in such a way that the fish will not be disturbed by persons continually walking

---

[1] Mention of trade names does not constitute endorsement.
[2] Duro-Test, Inc., Hammond, Ind.
[3] Sylvania, Inc., New York, N. Y.

past the system. Tanks and chambers must also be shielded from extraneous light which can affect the intended photoperiod or damage light sensitive eggs and alevins.

13. Construction materials: Construction materials which contact the diluent water should not contain leachable substances and should not sorb significant amounts of substances from the water. Stainless steel is probably the preferred construction material. Glass absorbs some trace organics significantly. Rubber should not be used. Plastic containing fillers, additives, stabilizers, plasticizers, etc., should not be used. Teflon, nylon, and their equivalents should not contain leachable materials and should not sorb significant amounts of most substances. Unplasticized polyethylene and polypropylene should not contain leachable substances, but may sorb very significant amounts of trace organic compounds.

14. Water: The water used should be from a well or spring if at all possible, or alternatively from a surface water source. Only as a last resort should water from a chlorinated municipal water supply be used. If it is thought that the water supply could be conceivably contaminated with fish pathogens, the water should be passed through an ultraviolet or similar sterilizer immediately before it enters the test system.

B. Biological system

1. Test animals: Yearling fish should be collected no later than March 1 and acclimated in the laboratory to test temperature and water quality for at least one month before the test is initiated. Suitability of fish for testing should be judged on the basis of acceptance of food, apparent lack of diseases, and 2% or less mortality during acclimation with no mortality two weeks prior to test. Set aside enough fish to supply an adequate number for short-term bioassay exposures used in determining application factors.

2. Beginning test: Begin exposure no later than April 1 by distributing 12 acclimated yearling brook trout per duplicate using a stratified random assignment (see D.3). This allows about a four month exposure to the toxicant before the onset of secondary or rapid growth phase of the gonads.

   Extra test animals may be added at the beginning so that fish can be removed periodically for special examinations (see B.12), or for residue analysis (see C.4).

3. Food: Use a good frozen trout food (e.g., Oregon Moist). Fish should be fed the largest pellet they will take a minimum of two times daily. The amount should be based on a reliable hatchery feeding schedule. Alevins and early juveniles should be fed trout starter a minimum of five times daily. Each batch of prepared food should be checked for pesticides (including DDT, TDE, dieldrin, endrin, aldrin,

BHC, chlordane, toxaphene, 2,4-D, and PCBs), and the kinds and amounts should be reported to the project officer or recorded.

4. Disease: Handle disease outbreaks according to their nature, with all tanks receiving the same treatment whether there seems to be sick fish in all of them or not. The frequency of treatment should be held to a minimum.

5. Measuring fish: Record mortalities daily, and measure fish directly at initiation of test, after three months and at thinning (see B.6) (total length and weight). Fish should not be fed 24 hours before weighing and lightly anesthetized with MS-222 to facilitate measuring (100 mg MS-222/liter water).

6. Thinning: When secondary sexual characteristics are well developed (approximately two weeks prior to expected spawning), separate males, females and undeveloped fish in each duplicate and randomly reduce sexually mature fish (see D.4) to the desired number of 2 males and 4 females, and discard undeveloped fish after examination. Place two spawning substrates (described earlier) in each duplicate. Record the number of mature, immature, deformed and injured males and females in each tank and the number from each category discarded. Measure total length and weight of all fish in each category before any are discarded and note which ones were discarded (see C.4).

7. Removing eggs: Remove eggs from the redd at a fixed time each day (preferably after 1:00 p.m. Evansville time, so the fish are not disturbed during the morning).

8. Egg incubation and viability: Impartially select 50 eggs from the first eight spawnings of 50 eggs or more in each duplicate and place them in an egg incubator cup for hatch. The remaining eggs from the first eight spawnings (>50 eggs) and all subsequent eggs from spawnings should be counted and placed in separate egg incubator cups for determining viability (formation of neural keel after 11-12 days at 9° C). The number of dead eggs from each spawn removed from the nest should be recorded and discarded. Never place more than 250 eggs in one egg incubator cup. All eggs incubated for viability are discarded after 12 days. Discarded eggs can be used for residue analysis and physiological measurements of toxicant related effects.

9. Progeny transfer: Additional important information on hatchability and alevin survival can be gained by transferring control eggs immediately after spawning to concentrations where spawning is reduced or absent, or to where an affect is seen on survival of eggs or alevin, and by transferring eggs from these concentrations to the control tanks. Two growth chambers for each duplicate spawning tank should always be reserved for eggs produced in that tank.

10. Hatch and alevin thinning: Remove dead eggs daily from the hatchability cups described in Step 8 above. When hatching commences, record the number hatched daily in each cup. Upon completion of hatch in any cup, randomly (see D.4) select 25 alevins from that cup. Dead or deformed alevins must not be included in the random selection but should be counted as being dead or deformed upon hatch. Measure total lengths of the 25 selected and discarded alevins. Total lengths are measured by the photographic method used by McKim and Benoit (1971). The fish are transferred to a glass box containing 1 inch of test water. They should be moved to and from this box in a water filled container, rather than by netting them. The glass box is placed on a translucent millimeter grid over a fluorescent light box which provides background illumination. Photos are then taken of the fish over the millimeter grid and are enlarged into 8 X 10 inch prints. The length of each fish is subsequnetly determined by comparing it to the grid. Keep lengths of discarded alevins separate from those which are kept. Place the 25 selected alevins back into the incubator cup and preserve the discarded ones for initial weights.

11. Alevin-juvenile exposure: Randomly (see D.4) select from the incubation cups two groups of 25 alevins each per duplicate for 90-day growth and survival exposures in the growth chambers. Hatching from one spawn may be spread out over a 3 to 6 day period; therefore, the median-hatch date should be used to establish the 90-day growth and survival period for each of the two groups of alevin. If it is determined that the median-hatch dates for the five groups per duplicate will be more than three weeks apart, then the two groups of 25 alevin must be selected from those which are less than three weeks old. The remaining groups in the duplicate which do not hatch during the three week period are used only for hatchability results and then photographed for lengths and preserved for initial weights. In order to equalize the effects of the incubation cups on growth, all groups selected for the 90-day exposure must remain in the incubation cups three weeks before they are released into the growth chambers. Each of the two groups selected per duplicate must be kept separate during the 90-day period. Record mortalities daily, along with total lengths 30 and 60 days post-hatch and total length and weight at 90 days post-hatch. Alevins and early juveniles should not be fed 24 hours before weighing. Total lengths are measured by transferring the growth chambers described earlier to a translucent millimeter grid over a fluorescent light box for photographing as described in Step 10 above. Survival and growth studies should be terminated after three months. Terminated fish can be used for tissue residue analysis and physiological measurements of toxicant related effects.

12. Parental termination: All parental fish should be terminated when a three week period passes in which no spawning occurs in any of the spawning tanks. Record mortality and weigh and measure total length of parental fish, check sex and condition of gonads (e.g., reabsorption, degree of maturation, spent ovaries, etc.) (see C.4).

13. Special examinations: Fish and eggs obtained from the test should be considered for physiological, biochemical, and histological investigations which may indicate certain toxicant related effects.

14. Necessary data: Data that must be reported for each tank of a chronic test are:

    a. Number and individual weights and total lengths of normal, deformed, and injured mature and immature males and females at initiation of test, three months after test commences, at thinning and at the end of test.

    b. Mortality during the test.

    c. Number of spawns and eggs. A mean incubation time should be calculated using date of spawning and the median hatch dates.

    d. Hatchability.

    e. Fry survival, growth and deformities.

C. Chemical system

1. Preparing a stock solution: If a toxicant cannot be introduced into the test water as is, a stock solution should be prepared by dissolving the toxicant in water or an organic solvent. Acetone has been the most widely used solvent, but dimethylformanide (DMF) and triethylene glycol may be preferred in many cases. If none of these solvents are acceptable, other water-miscible solvents such as methanol, ethanol, isopropanol, acetonitrile, dimethyl-acetamide (DMAC), 2-ethoxyethanol, glyme (dimethylether of ethylene glycol, diglyme (dimethyl ether of diethylene glycol) and propylene glycol should be considered. However, dimethyl sulfoxide (DMSO) should not be used if at all possible because of its biological properties.

   Problems of rate of solubilization or solubility limit should be solved by mechanical means if at all possible. Solvents, or as a last resort, surfactants, can be used for this purpose, only after they have been proven to be necessary in the actual test

system. The suggested surfactant is p-tert-octylphenoxynonaethoxyethanol (p-1, 1, 3, 3-tetramethylbutylphenoxynonaethoxyethanol, $OPE_{10}$) (Triton X-100, a product of the Rohm and Haas Company, or equivalent).

The use of solvents, surfactants, or other additives should be avoided whenever possible. If an additive is necessary, reagent grade or better should be used. The amount of an additive used should be kept to a minimum, but the calculated concentration of a solvent to which any test organisms are exposed must never exceed one one-thousandth of the 96-hr. TL50 for test species under the test conditions and must never exceed one gram per liter of water. The calculated concentration of surfactant or other additive to which any test organisms are exposed must never exceed one-twentieth of the concentration of the toxicant and must never exceed one-tenth gram per liter of water. If any additive is used, two sets of controls must be used, one exposed to no additives and one exposed to the highest level of additives to which any other organisms in the test are exposed.

2. Measurement of toxicant concentration: As a minimum the concentration of toxicant must be measured in one tank at each toxicant concentration every week for each set of duplicate tanks, alternating tanks at each concentration from week to week. Water samples should be taken about midway between the top and bottom and the sides of the tank and should not include any surface scum or material stirred up from the bottom or sides of the tank. Equivolume daily grab samples can be composited for a week if it has been shown that the results of the analysis are not affected by storage of the sample.

   Enough grouped grab samples should be analyzed periodically throughout the test to determine whether or not the concentation of toxicant is reasonably constant from day to day in one tank and from one tank to its duplicate. If not, enough samples must be analyzed weekly throughout the test to show the variability of the toxicant concentration.

3. Measurement of other variables: Temperature must be recorded continuously (see A.11).

   Dissolved oxygen must be measured in the tanks daily at least five days a week on an alternating basis, so that each tank is analyzed once each week. However, if the toxicant or an additive causes a depression in dissolved oxygen, the toxicant concentration with the lowest dissolved oxygen concentration must be analyzed daily in addition to the above requirement.

   A control and one test concentration must be analyzed weekly for pH, alkalinity, hardness, acidity, and conductance or more often,

if necessary, to show the variability in the test water. However, if any of these characteristics are affected by the toxicant, the tanks must be analyzed for that characteristic daily, at least five days a week, on an alternating basis, so that each tank is analyzed once every other week.

At a minimum, the test water must be analyzed at the beginning and near the middle of the chronic test for calcium, magnesium, sodium, potassium, chloride, sulfate, conductance, total solid, and total dissolved solids.

4. Residue analysis: When possible and deemed necessary, mature fish, and possibly eggs, larvae, and juveniles, obtained from the test, should be analyzed for toxicant residues. For fish, muscle should be analyzed, and gill, blood, brain, liver, bone kidney, GI tract, gonad, and skin should be considered for analysis. Analyses of whole organisms may be done in addition to, but should not be done in place of, analyses of individual tissues, especially muscle.

5. Methods: When they will provide the desired information with acceptable precision and accuracy, methods described in Methods for Chemical Analysis of Water and Wastes (EPA, 1971) should be used unless there is another method which requires much less time and can provide the desired information with the same or better precision and accuracy. At a minimum, accuracy should be measured using the method of known additions for all analytical methods for toxicants. If available, reference samples should be analyzed periodically for each analytical method.

D. Statistics

1. Duplicates: Use true duplicates for each level of the toxic agent, i.e., no water connections between duplicate tanks.

2. Distribution of tanks: The tanks should be assigned to locations by stratified random assignment (random assignment of one tank for each level of the toxic agent in a row followed by random assignment of the second tank for each level of the toxic agent in another or an extension of the same row).

3. Distribution of test organisms: The test organisms should be assigned to tanks by stratified random assignment (random assignment of one test organism to each tank, random assignment of a second test organism to each tank, etc.).

4. Selection and thinning test organisms: At time of selection or thinning of test organisms the choice must be random (random, as defined statistically).

E. Miscellaneous

1. Additional information: All routine bioassay flow through methods not covered in this procedure (e.g., physical and chemical determinations, handling of fish) should be followed as described in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1971).

2. Acknowledgments: These procedures for the brook trout were compiled by J. M. McKim and D. A. Benoit for the Committee on Aquatic Bioassays. The participating members of this committee are: Robert Andrew, John Arthur, Duane Benoit, Gerald Bouck, William Brungs, Gary Chapman, John Eaton, John Hale, Kenneth Hokanson, James McKim, Quentin Pickering, Wesley Smith, Charles Stephan, and James Tucker.

3. References: For additional information concerning flow through bioassay tests with brook trout, the following references are listed:

Allison, L. N. 1951. Delay of spawning in eastern brook trout by means of artificially prolonged light intervals. Progressive Fish-Culturist, 13: 111-116.

American Public Health Association. 1971. Standard methods for the examination of water and wastewater. 13th ed. APHA, New York.

Carson, B. W. 1955. Four years progress in the use of artificially controlled light to induce early spawning of brook trout. Progressive Fish-Culturist, 17: 99-102.

Drummond, Robert A., and Walter F. Dawson. 1970. An inexpensive method for simulating Diel patterns of lighting in the laboratory. Trans. Amer. Fish. Soc., 99(2): 434-435.

Environmental Protection Agency. 1971. Methods for Chemical Analysis of Water and Wastes. Analytical Quality Control Laboratory, Cincinnati, Ohio.

Fabricius, E. 1953. Aquarium observations on the spawning behavior of the char, Salmo alpinus. Rep. Inst. Freshwater Res. Drottingholm, 34: 14-48.

Hale, J. G. 1968. Observations on brook trout, Salvelinus fontinalis spawning in 10-gallon aquaria. Trans. Amer. Fish. Soc.. 97: 299-301.

Henderson, N. E. 1962. The annual cycle in the testis of the eastern brook trout, Salvelinus fontinalis (Mitchill) Canadian Jour. Zool., 40: 631-645.

Henderson, N. E. 1963. Influence of light and temperature on the reproductive cycle of the eastern brook trout Salvelinus fontinalis (Mitchill). J. Fish. Res. Bd. Canada, 20(4): 859-897.

Hoover, E. E., and H. E. Hubbard. 1937. Modification of the sexual cycle in trout by control of light. Copeia, 4: 206-210.

MacFadden, J. 1961. A population study of the brook trout Salvelinus fontinalis (Mitchill). Wildlife Soc. Pub. No. 7.

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

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

Mount, Donald I., and William Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Research, 1: 21-29.

Pyle, E. A. 1969. The effect of constant light or constant darkness on the growth and sexual maturity of brook trout. Fish. Res. Bull. No. 31. The nutrition of trout, Cortland Hatchery Report No. 36, pages 13-19.

Wydoski, R. S., and E. L. Cooper. 1966. Maturation and fecundity of brook trout from infertile streams. J. Fish. Res. Bd. Canada, 23(5): 623-649.

Approved by the Committee on
Aquatic Bioassays, NWQL

Approved by the Director, NWQL

## Appendix A

Test (Evansville, Indiana) Photoperiod

For Brook Trout Partial Chronic

| Dawn to Dusk Time | Date | | Day-length (hour and minute) | |
|---|---|---|---|---|
| 6:00 - 6:15) | MAR. | 1 | 12:15) | |
| 6:00 - 7:00) | | 15 | 13:00) | |
| | | | ) | |
| 6:00 - 7:30) | APR. | 1 | 13:30) | |
| 6:00 - 8:15) | | 15 | 14:15) | |
| | | | ) | |
| 6:00 - 8:45) | MAY | 1 | 14:45) | |
| 6:00 - 9:15) | | 15 | 15:15) | |
| | | | ) | |
| 6:00 - 9:30) | JUNE | 1 | 15:30) | Juvenile- |
| 6:00 - 9:45) | | 15 | 15:45) | adult exposure |
| | | | ) | |
| 6:00 - 9:45) | JULY | 1 | 15:45) | |
| 6:00 - 9:30) | | 15 | 15:30) | |
| | | | ) | |
| 6:00 - 9:00) | AUG. | 1 | 15:00) | |
| 6:00 - 8:30) | | 15 | 14:30) | |
| | | | ) | |
| 6:00 - 8:00) | SEPT. | 1 | 14:00) | |
| 6:00 - 7:30) | | 15 | 13:30) | |
| | | | | |
| 6:00 - 6:45) | OCT. | 1 | 12:45) | |
| 6:00 - 6:15) | | 15 | 12:15) | |
| | | | ) | Spawning and |
| 6:00 - 5:30) | NOV. | 1 | 11:30) | egg incubation |
| 6:00 - 5:00 | | 15 | 11:00) | |
| | | | | |
| 6:00 - 4:45) | DEC. | 1 | 10:45) | |
| 6:00 - 4:30) | | 15 | 10:30) | |
| | | | ) | |
| 6:00 - 4:30) | JAN. | 1 | 10:30) | Alevin-juvenile |
| 6:00 - 4:45) | | 15 | 10:45) | exposure |
| | | | ) | |
| 6:00 - 5:15) | FEB. | 1 | 11:15) | |
| 6:00 - 5:45) | | 15 | 11:45) | |

## Appendix B

Temperature Regime for Brook Trout Partial Chronic

| Months | | Temperature ° C |
|---|---|---|
| Mar. | Juvenile-adult exposure | 9 |
| Apr. | | 12 |
| May | | 14 |
| June | | 15 |
| July | | 15 |
| Aug. | | 15 |
| Sept. | | 12 |
| Oct. | Spawning and egg incubation | 9 |
| Nov. | | 9 |
| Dec. | Alevin-juvenile exposure | 9 |
| Jan. | | 9 |
| Feb. | | 9 |
| Mar. | | 9 |

A constant temperature must be established just prior to spawning and egg incubation, and maintained throughout the 3-month alevin-juvenile exposure.

## TECHNICAL REPORT DATA

*(Please read Instructions on the reverse before completing)*

| 1. REPORT NO. | 2. | 3. RECIPIENT'S ACCESSION NO. |
|---|---|---|
| EPA-600/3-76-112 | | |

**4. TITLE AND SUBTITLE**
EFFECTS OF AROCLOR® 1254 ON BROOK TROUT, SALVELINUS FONTINALIS

**5. REPORT DATE**
December 1976

**6. PERFORMING ORGANIZATION CODE**

**7. AUTHOR(S)**
Virginia M. Snarski and Frank A. Puglisi

**8. PERFORMING ORGANIZATION REPORT NO.**

**9. PERFORMING ORGANIZATION NAME AND ADDRESS**
Environmental Research Laboratory - Duluth, MN
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804

**10. PROGRAM ELEMENT NO.**
1BA608

**11. CONTRACT/GRANT NO.**
None (in-house)

**12. SPONSORING AGENCY NAME AND ADDRESS**
Same as above

**13. TYPE OF REPORT AND PERIOD COVERED**
Final 1972-1974

**14. SPONSORING AGENCY CODE**
EPA/600/03

**15. SUPPLEMENTARY NOTES**

**16. ABSTRACT**

No adverse effects were observed on survival, growth, and reproduction of brook trout exposed for 71 weeks to 0.94 µg/1. and lower concentrations of the polychlorinated biphenyl Aroclor® 1254 (P = 0.05). Survival and growth to 90 days of alevin-juveniles from exposed parents were also unaffected ( P = 0.05). Polychlorinated biphenyl concentrations in the brook trout were directly proportional to the water exposure concentrations (P = 0.05). The PCB tissue concentrations appeared to have reached a steady state by the first sampling after 14 weeks of exposure. The PCB residues (wet-tissue basis) in chronically exposed fish were approximately 2 µg/g in the fillet and 9 µg/g in the "whole body" (entire fish minus one fillet and the gonads) at the highest water concentration, 0.94 µg/1. The higher residue in the whole body compared to the corresponding fillet was due to the higher fat content of the former.

**17. KEY WORDS AND DOCUMENT ANALYSIS**

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| Bioassay<br>Trout<br>Survival<br>Growth<br>Reproduction<br>Freshwater<br>Tissues<br>Chlorohydrocarbon | Aroclor® 1254<br>Polychlorinated biphenyl<br>biphenyl<br>Bioaccumulation | 06/F/T |

| 18. DISTRIBUTION STATEMENT | 19. SECURITY CLASS (This Report) | 21. NO. OF PAGES |
|---|---|---|
| RELEASE TO PUBLIC | UNCLASSIFIED | 42 |
| | 20. SECURITY CLASS (This page) | 22. PRICE |
| | UNCLASSIFIED | |

EPA Form 2220-1 (9-73)



☆ U. S. GOVERNMENT PRINTING OFFICE 1977-757-056/5552 Region No. 5-11