



12-1978

Effects of Polychlorinated Biphenyl Compounds and Proposed PCB-Replacement Products on Embryo-Larval Stages of Fish and Amphibians

Digital Object Identifier: <https://doi.org/10.13023/kwrri.rr.118>

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EFFECTS OF POLYCHLORINATED BIPHENYL COMPOUNDS
AND PROPOSED PCB-REPLACEMENT PRODUCTS ON
EMBRYO-LARVAL STAGES OF FISH AND AMPHIBIANS

By

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Project Number: A-067-KY (Completion Report)
Agreement Numbers: 14-34-0001-7037 (FY 1976T)
14-34-0001-7038 (FY 1977)
14-34-0001-8019 (FY 1978)
Period of Project: July 1976 - September 1978

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The work on which this report is based was supported in part by funds provided by the Office of Water Research and Technology, United States Department of the Interior, as authorized under the Water Resources Research Act of 1964.

December 1978



ABSTRACT

Static renewal bioassays were performed with four polychlorinated biphenyls, including Capacitor 21 and Aroclors 1016, 1242, and 1254. Each compound was used to treat embryo-larval stages of the rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), goldfish (Carassius auratus), redear sunfish (Lepomis microlophus), leopard frog (Rana pipiens), Fowler's toad (Bufo fowleri), and American toad (Bufo americanus). Developmental stages of the trout were the most sensitive, with LC₅₀'s at 4 days posthatching of 0.3, 1.0, 1.1, and 1.6 µg/l for Aroclors 1254, 1242, 1016, and Capacitor 21, respectively. LC₁'s ranged between 0.009-0.011 µg/l. Fowler's toad was the most tolerant species, and LC₅₀'s varied from 3.7-28.0 µg/l. Toxicity of the PCB's generally increased with percent chlorine substitution.

Bioassays also were conducted with dioctylphthalate (DOP), diisononylphthalate, and Dow Corning 561 silicone, three products proposed as replacements for PCB's. Embryos and larvae of the channel catfish, redear sunfish, leopard frog, and Fowler's toad were exposed through 4 days posthatching. Catfish was the most sensitive species, and sunfish was the most resistant. Based on LC₅₀ values, replacement products were found to be 2-5 orders of magnitude less toxic than PCB's.

Continuous flow embryo-larval bioassays were performed on Capacitor 21 and DOP. Test species included redear sunfish, rainbow trout, and largemouth bass (Micropterus salmoides). Paralleling results from static renewal tests, flow-through data reflected a vast difference in toxicity between PCB's and potential replacements, and good data correlation was achieved between the two bioassay procedures.

Descriptors: Polychlorinated biphenyls
Dioctylphthalate
Diisononylphthalate
Polydimethylsiloxanes
Embryo-larval tests
Teratogenesis

ACKNOWLEDGMENTS

We are most appreciative for the assistance of Donald M. Bruser and William E. McDonnell in the analyses of organic compounds used in this study. We are also grateful to Barbara A. Ramey for preparation of the manuscript and figures. The research facilities used to conduct these tests were provided in part by research funds from the Environmental Protection Agency, Office of Toxic Substances (contract no. 68-01-4321).

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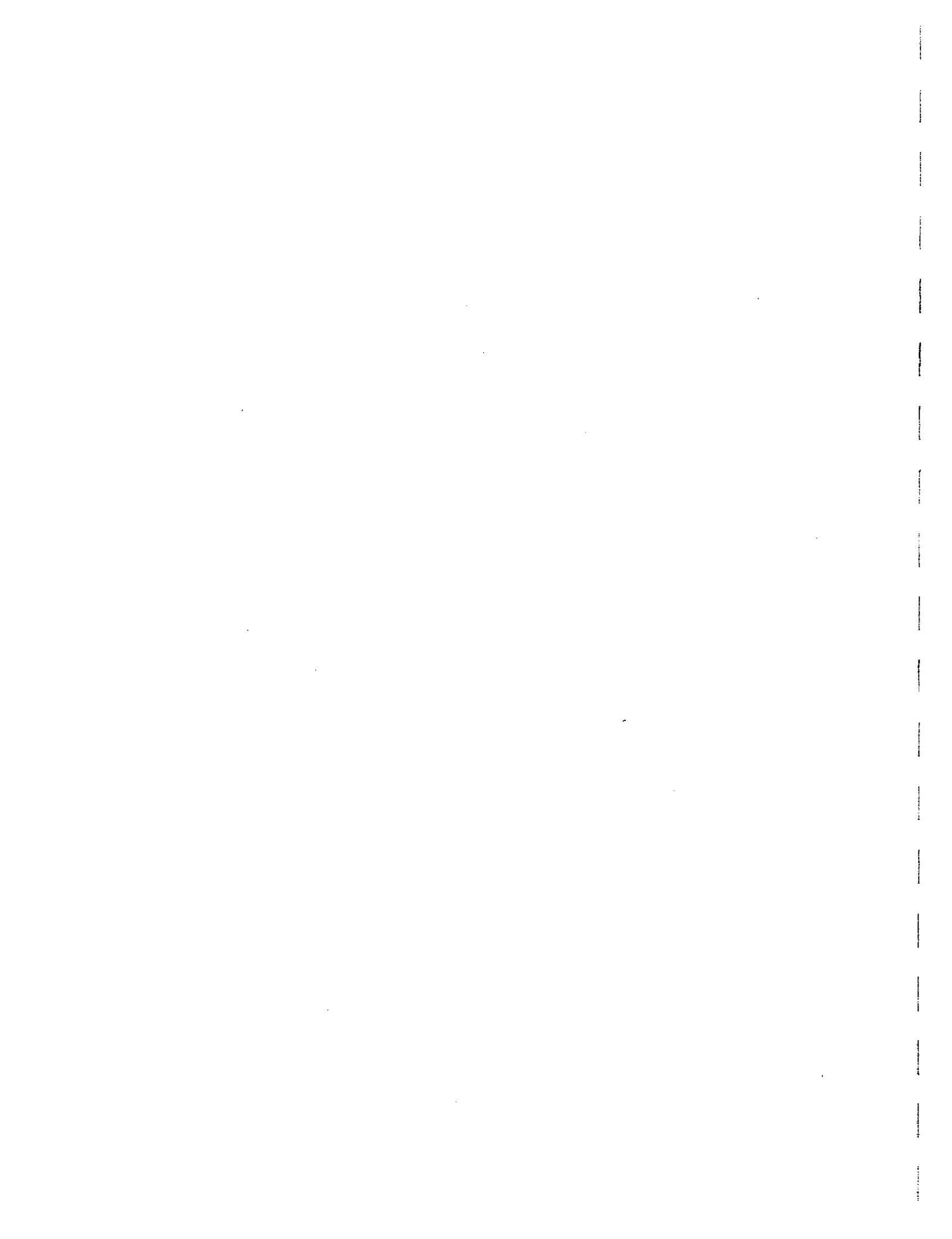
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CHAPTER I

INTRODUCTION

Polychlorinated biphenyl compounds (PCB's) have polluted nearly all the major waterways in the U.S., presenting an environmental problem rivaling those of mercury and DDT. Despite curtailment of Aroclor production in the U.S., PCB's still in use or disposed of in recent years are of considerable concern with respect to freshwater resources. This continuing problem is due to the large quantities of PCB's produced during the last decade and the long environmental half-life of such compounds. Distribution of PCB's is widespread in natural resources, and concentrations present in animal tissues are considerably greater than levels of either mercury or organochlorine pesticides (Nelson, 1972; NAS-NAE Committee, 1973). In addition to their propensity for bioaccumulation, PCB's are known to inhibit animal reproduction. In studies with avian eggs, Bowes, et al. (1973) have detected concentrations as high as 3700 $\mu\text{g/g}$ in lipid fractions, and these levels were correlated with embryonic death and reduced hatchability. PCB's also have been shown to affect adversely developmental stages of fish and certain invertebrates. For example, exposure to 1.3 $\mu\text{g/l}$ Aroclor 1254 for three weeks produced 50% reproductive impairment in cultures of Daphnia magna (Nebeker and Puglisi, 1974). In a chronic study with the fathead minnow, embryos and early fry were found to be the most sensitive life-cycle stages to several PCB compounds (Nebeker, et al., 1974). Jensen, et al. (1970) reported that PCB residues of 0.4-1.9 $\mu\text{g/g}$ in salmon eggs resulted in mortality ranging from 16% to 100%. Since the accumulation of PCB's in fish eggs and tissues may be 75,000-200,000 times the concentration found in water (Hansen, et al., 1971; NAS-NAE Committee, 1973; Environmental Protection Agency, 1976), environmental levels as low as 2-25 ng/l may inhibit embryonic development in salmonid fishes.

Based on the high bioaccumulation factors determined for PCB compounds and the FDA standard of 5 $\mu\text{g/g}$ for fish tissues, EPA has

established a criterion of 0.001 $\mu\text{g}/\text{l}$ for freshwater and marine aquatic life (Environmental Protection Agency, 1976). However, only a limited number of investigations have been conducted to determine the direct toxic effects of PCB's on developmental stages of fish, and virtually no such studies have been performed with amphibian species. In addition, there are only minimal data available on the aquatic toxicity of several chemicals being considered as replacements for PCB's in electrical capacitors and transformers. Therefore, the objective of this study was to evaluate and compare the effects of four PCB's (Aroclors 1254, 1242, 1016, Capacitor 21) and three proposed replacement compounds (diisononylphthalate, dioctylphthalate, Dow Corning 561 silicone transformer fluid) on developmental stages of fish and amphibians. Embryos and larvae were exposed to toxicants in both static renewal and flow-through systems to determine the concentrations which produce mortality and teratogenesis and to identify no-effect levels. As economic constraints limited utilization of more exhaustive procedures, static renewal bioassays were used initially to determine the relative toxicities of all seven compounds and to evaluate animal species for sensitivity. Selected toxicants were then tested using more involved flow-through bioassay procedures.

CHAPTER II

RESEARCH PROCEDURES

Animal species and test conditions. Studies were undertaken to determine the effects of PCB compounds and potential PCB-replacement products on embryonic and larval stages of fish and amphibians. The species selected for testing included the channel catfish (Ictalurus punctatus), goldfish (Carassius auratus), largemouth bass (Micropterus salmoides), rainbow trout (Salmo gairdneri), redear sunfish (Lepomis microlophus), American toad (Bufo americanus), Fowler's toad (Bufo fowleri), and leopard frog (Rana pipiens). Gravid rainbow trout were provided by the Erwin National Fish Hatchery, Erwin, Tennessee. Eggs and sperm were obtained using the spawning and milking procedures of Leitritz and Lewis (1976). For all other test species, freshly fertilized eggs were collected locally from the Frankfort National Fish Hatchery, Frankfort, Kentucky. Bioassay treatment was initiated 15 minutes after fertilization in trout and 2-6 hours after spawning in other organisms. Exposure was maintained continuously through 4 days posthatching. Average hatching times were 22 days for the trout (13.5-14.3°C), 3 days for catfish (29-31°C), and 3-4 days for the remaining species (20-24°C).

All aquatic bioassays were performed in temperature-regulated environmental rooms. Control eggs were cultured simultaneously with experimentals and under identical conditions, except for omission of toxicants. Eggs were examined daily to gauge extent of development and to remove dead specimens. Sample size ranged from 100-200 eggs per exposure concentration. Percent survival was expressed as frequency in the experimental population/controls and was determined at hatching and 4 days posthatching. Although bioassays initially were extended through 8 days, larval mortality was usually insignificant after the first several days. For that reason, subsequent tests were terminated at

4 days posthatching, and the latter time was used as an end point for data reporting. In all instances, survival frequencies were based on accumulative test responses incurred from onset of treatment. Teratogenesis was determined at hatching and expressed as percent of survivors affected by gross debilitating anomalies (Birge and Black, 1977a). Counting teratic animals as lethals, log probit analysis (Finney, 1971) was used to determine LC_{50} and LC_1 values with 95% confidence limits. There were several tests for which reliable LC_1 values could not be determined using the probit computer program. In these cases, the LC_1 was interpolated from a dose-response graph, and confidence limits are represented by dashes on the data tables.

Reconstituted water was prepared by the addition of reagent grade calcium, magnesium, sodium, and potassium salts to distilled, double deionized water. Physicochemical characteristics of the test water are given in Table 1. Concentrations of cations and anions were within ranges published for freshwater resources in Arizona (Dutt and McCreary, 1970), Kentucky (U.S. Geological Survey, 1972), and other areas of the U.S. (McKee and Wolf, 1963; Mount, 1968). Total chloride content, total dissolved solids, and the concentration of sodium plus potassium were under maximum levels of 170 mg/l, 400 mg/l, and 85 mg/l, observed for 95% of U.S. waters found to support a good, mixed fish fauna (Hart, *et al.*, 1945). Specific conductivity compared favorably with values of 150-500 μ mhos/cm recommended for fish propagation (McKee and Wolf, 1963), and osmolarity was well under the maximum limit of 50 mOsm/Kg water suggested for U.S. freshwaters (National Technical Advisory Committee, 1968). Total alkalinity and pH were within optimum ranges for aquatic habitat (Baas Becking, *et al.*, 1960; McKee and Wolf, 1963; National Technical Advisory Committee, 1968).

Test compounds. The polychlorinated biphenyls tested were Aroclors 1016, 1242, 1254, and Capacitor 21, all obtained through the courtesy of the Monsanto Company, St. Louis, Missouri. The replacement products included dioctylphthalate (DOP), diisononylphthalate (DINP), and Dow Corning 561 silicone transformer fluid (DC 561) and were provided by the United States Steel Chemicals Division, Pittsburgh,

Table 1. Reconstituted test water.

Hardness as CaCO ₃ :	50 mg/l	100 mg/l	200 mg/l
DISSOLVED SALTS ¹ , mg/l			
CaCl ₂	37.5	75.0	150
MgSO ₄ ·7H ₂ O	37.5	75.0	150
NaHCO ₃	100	100	100
KCl	5	5	5
CHEMICAL COMPOSITION, mg/l			
Ca	13.6	27.1	54.2
Mg	3.7	7.4	14.8
Na	27.4	27.4	27.4
K	2.6	2.6	2.6
Cl	26.3	52.3	98.2
HCO ₃	72.6	72.6	72.6
SO ₄	14.6	29.2	58.5
PHYSICOCHEMICAL CHARACTERISTICS ²			
Hardness, as mg/l CaCO ₃	53.3 ± 1.3	101.6 ± 4.4	197.5 ± 5.8
pH	7.84 ± 0.02	7.70 ± 0.01	7.78 ± 0.02
Total alkalinity, as mg/l CaCO ₃	66.7 ± 0.4	65.0 ± 0.4	65.3 ± 0.6
Conductivity, μmhos/cm	133.6 ± 1.4	176.0 ± 1.0	282.0 ± 1.9
Osmolarity, mOsm/Kg H ₂ O	8.9 ± 0.2	10.8 ± 0.3	12.7 ± 0.4
Total dissolved solids, mg/l	121.4 ± 4.4	171.8 ± 2.0	336.7 ± 7.8
Dissolved oxygen, mg/l at 13.5°C	9.9 ± 0.2	10.1 ± 0.2	10.1 ± 0.2

¹Prepared in distilled, deionized water with a specific conductivity of 0.25 μmhos or less.

²Measurements made at 25°C except where noted. Mean with standard error determined for 10 replicates.

Pennsylvania; the Exxon Chemical Company, Houston, Texas; and the Dow Corning Corporation, Midland, Michigan, respectively.

Static renewal bioassays. Static renewal embryo-larval bioassays were performed on all seven compounds, using the technique of Birge and Just (1975). Depending on toxicant concentration, acetone at 0.04 $\mu\text{g/l}$ to 4.0 mg/l was used as the carrier solvent. Before each 12-hour renewal interval, stock solutions of the test compound, acetone, and water were homogenized in high speed blenders to facilitate mixing. Exposures were based on initial toxicant concentrations. Both acetone-treated and acetone-free controls were maintained simultaneously with experimental populations. Control eggs receiving acetone treatment gave hatching frequencies of 88-99%, compared to 94-99% for untreated embryos. This difference was not significant ($P > 0.05$, t test).

Fish and amphibian eggs were cultured in 500-ml deep Petri dishes and test water was monitored for water hardness, pH, and dissolved oxygen by procedures described below under Flow-Through Bioassays. Using moderate, continuous aeration, dissolved oxygen was maintained near saturation, and pH ranged from 7.6-8.1. The water hardness selected for static renewal bioassays was moderately hard, with an overall range of 90-115 mg/l CaCO_3 .

Flow-through bioassays. Flow-through bioassays were conducted with Capacitor 21 and DOP in both soft and hard water (*i.e.*, 50 and 200 mg/l CaCO_3). The flow system, designed for testing volatile and insoluble organic compounds without the use of carrier solvents, is illustrated in Figure 1. Toxicant was administered to a mixing chamber situated ahead of each egg chamber, using graduated flow from a syringe pump (Sage Instruments, model 355). Test water was delivered to the mixing chamber by regulated flow from a peristaltic pump (Brinkmann Instruments, Inc., model 131900). Solutions from the two pump channels were mixed by mechanical stirring or homogenization, and delivered from the mixing unit to the test chamber under positive pressure at 200 ml/hr . Toxicant exposure was regulated by adjusting the mixing ratio between pumping units and/or by varying the concentration of toxicant delivered from the syringe pump. Syringe pumps were fitted with modified holders, and each unit was operated using up to six double-ground glass syringes.

Test chambers were constructed from 3" Pyrex pipe joints, provided with clamp-locking O-ring seals. Using standard glass blowing techniques, the pipe was cut and sealed to give a test chamber capacity of 500 ml (Figure 2). An outlet tube was annealed to the cover, and an inlet was positioned near the bottom of the chamber. A stainless steel inlet screen was positioned 3 cm from the bottom of the dish, dividing the chamber into upper egg and lower stirring compartments. Fish eggs were supported on the inlet screen, and a Teflon-coated magnetic stirring bar was used in the lower compartment to provide moderate, continuous agitation of test water. An upper outlet screen was used to retain hatched organisms. The outlet screen was held in place by a Pyrex pedestal, and the inlet screen was supported by a constriction on the wall of the stirring compartment (Figure 2). Access to test organisms was obtained by opening the water-tight joint and removing the chamber cover. Prior to opening the chamber, a rapid-disconnect was used to remove the inlet line and to drain the fluid level down to the O-ring seal. When perfused with a continuous flow of oxygen-saturated water, the sealed chamber was essentially free of standing air space.

High speed homogenization of toxicants in test water was accomplished using an Oster blender, equipped with a 400-ml glass container. The latter was provided with inlets for syringe and peristaltic pump lines and a side outlet for supply of water-toxicant homogenate to the test chamber (Figures 2.1, 2.2). Pyrex tubing (3 mm O.D.) was used to extend pump inlet lines to a depth of 3 cm above the stirring blades. Blending time was regulated with an electronic timer. Moderate agitation supplied to the bioassay chamber and regulation of flow rate were used to prevent the compounds from partitioning out of test water.

Test water was monitored daily for temperature, dissolved oxygen, water hardness, and pH, using a YSI tele-thermometer with thermocouple, YSI oxygen meter (model 51A), Orion divalent cation electrode, and a Corning digital pH meter (model 110). Flow rates from peristaltic pumps, monitored twice daily with liquid flow meters (Gilmont, Inc.), varied from 180-220 ml/hr. The two levels of water hardness were 45-55 and

190-225 mg/l CaCO_3 , and pH ranged from 7.5-8.0. Continuous aeration, supplied to peristaltic pump reservoirs, maintained dissolved oxygen at 85-98% of saturation. Survival of control organisms ranged from 90-99%.

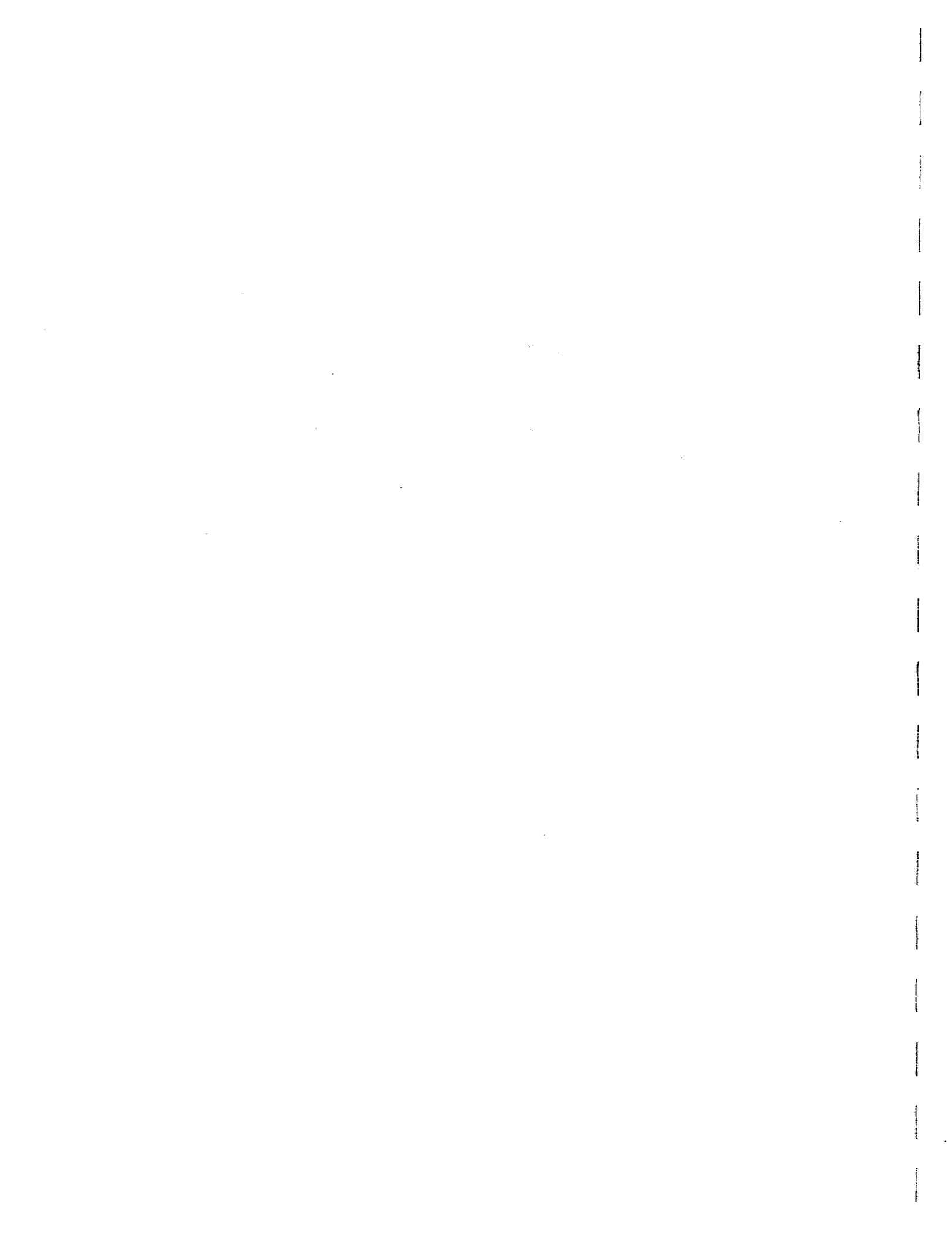
In the flow-through bioassays, exposure concentrations were confirmed by daily analysis of test water. Capacitor 21 was extracted from 0.5-2.0 liter aliquots of test water, using reagent grade chloroform. Chloroform extracts were dried with anhydrous sodium sulfate and concentrated by evaporation. Capacitor 21 was quantified using a Packard gas chromatograph (model 7400), equipped with an electron capture detector and a glass column (2 m X 2 mm I.D.). The stationary phase was 3% Dexsil 300 GC (Alltech Associates, Inc.) on 80/100 Chromosorb WAW (Supelco, Inc.), with oven, inlet, and detector temperatures of 220°, 250°, and 260°C, respectively. Nitrogen carrier gas was used at a flow rate of 50 ml/min. Extracted samples were compared to Capacitor 21 standards prepared in ethyl acetate, and the detection limit was 0.025 µg/l.

Diethylphthalate was extracted with reagent grade chloroform from 0.25-2.0 liter aliquots of test water. The extract was evaporated to dryness and the residue was reconstituted in ethyl acetate. DOP was analyzed by gas chromatography, using a flame ionization detector and a glass column (0.5 m X 2 mm I.D.). The stationary phase was 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb WHP (Supelco, Inc.). Oven, inlet, and detector temperature were 250°, 265°, and 280°C, respectively. Nitrogen was used as the carrier gas at 50 ml/min. The extracted samples were compared to DOP standards prepared in ethyl acetate, and the detection limit was 0.01 mg/l.

FIGURE 1

Flow-through Bioassay System for Fish Embryo-larval Stages

Peristaltic pumps (A) and syringe pumps (B) were used to supply diluent water and toxicant to mixing chambers (C and D). Water and toxicant were blended with homogenizers (C) or magnetic stirrers (D) and delivered under positive pressure to test chambers (E). The multi-channel system was maintained in an environmental room and syringe pumps were mounted on the outside wall to avoid effects of high humidity on operation.



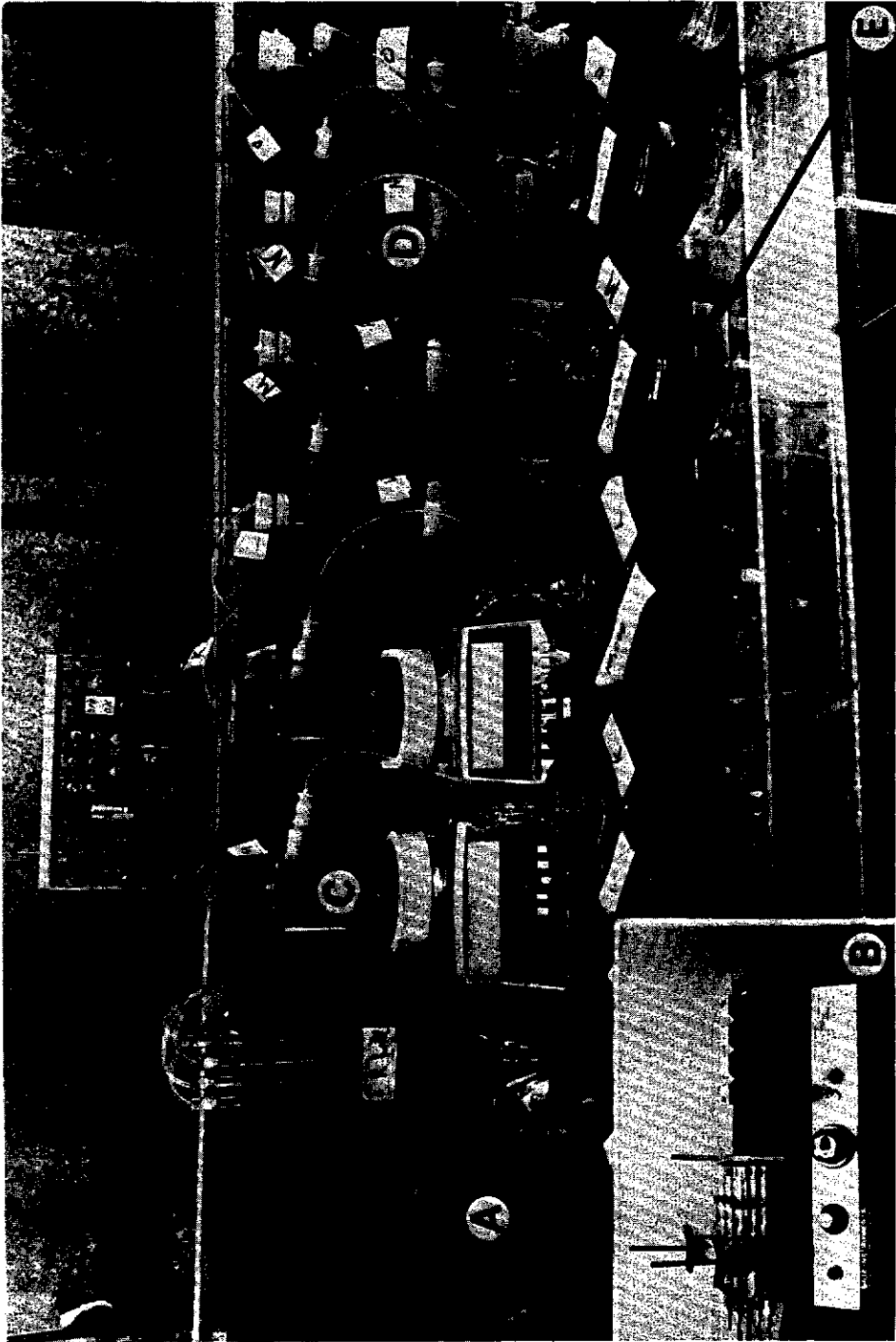
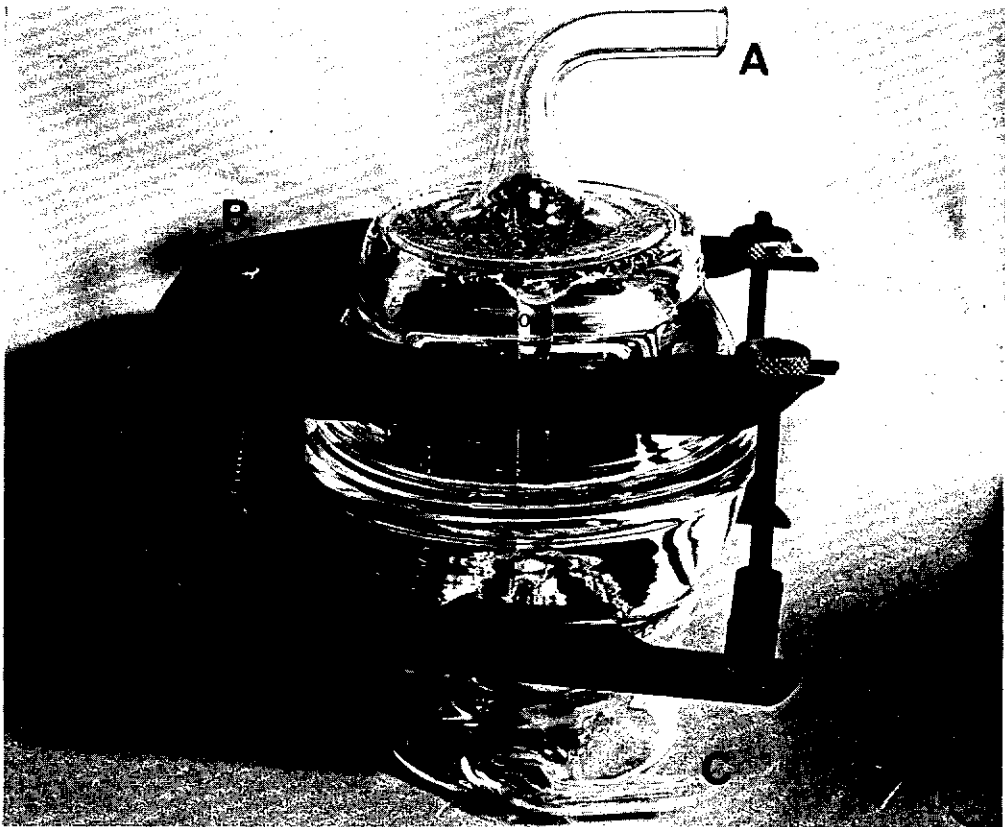
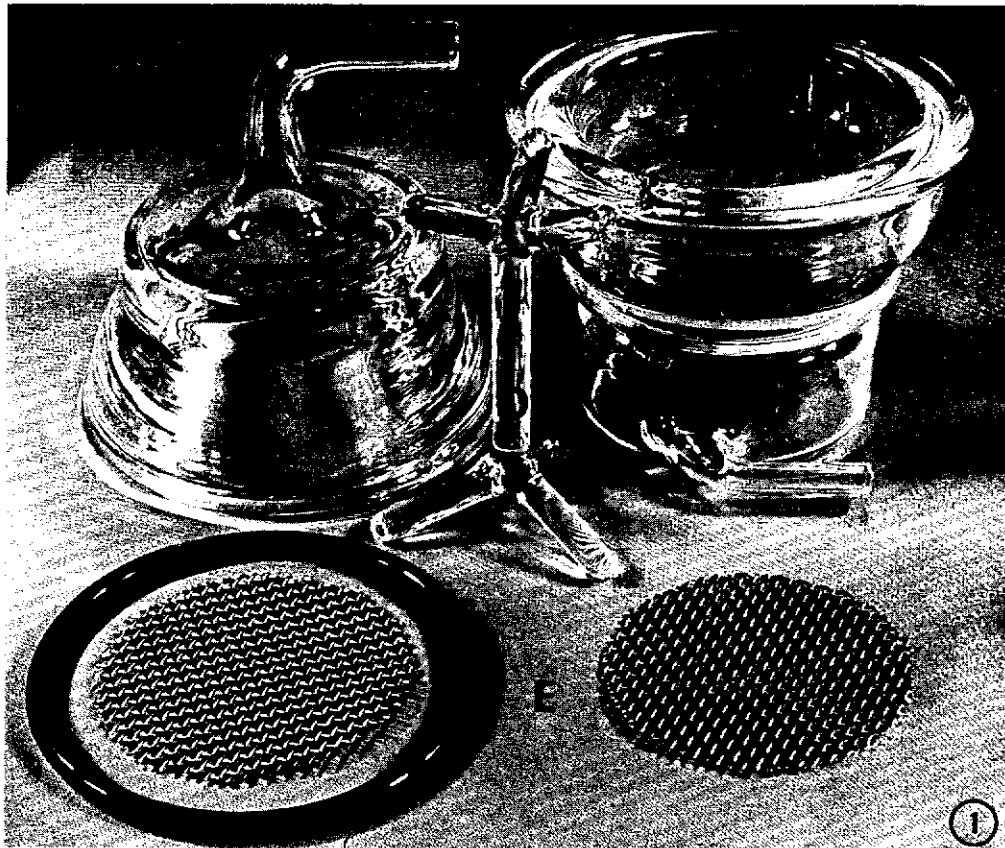


FIGURE 2

Exposure Chamber

- 2.1 Disassembled chamber, including cover (A), egg compartment (B), stirring compartment (C), screen support (D), and O-ring with inlet and outlet screens (E).
- 2.2 Assembled test chamber, showing outlet from egg compartment (A), locking clamp (B), and stirring compartment inlet (C).



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CHAPTER III

RESULTS AND DISCUSSION

Static renewal bioassays. Polychlorinated biphenyls were administered to embryos and larvae of seven fish and amphibian species. Each compound was tested at five concentrations, starting at 0.1 $\mu\text{g}/\text{l}$ and increasing at 10-fold intervals to 1 mg/l . Toxicity data summarized in Table 2 include both normal and defective animals which survived through hatching and 4 days posthatching. The frequencies of teratic individuals in hatched populations are given parenthetically. However, calculations for LC_{50} and LC_1 values (Tables 4-6) included grossly anomalous larvae as lethals.

Aroclor 1254 at 0.1 $\mu\text{g}/\text{l}$ reduced the hatchability of trout eggs by 20% compared to control populations, and 10 $\mu\text{g}/\text{l}$ produced complete mortality. Similar toxicity was observed for the sunfish. These results compared closely with data reported in tests with embryo-larval stages of other piscine species. For example, exposure to 0.32 $\mu\text{g}/\text{l}$ Aroclor 1254 was found to reduce survival of early fry of the sheepshead minnow to 62%, compared to 89% for controls (Schimmel, et al., 1974). With the fathead minnow, 46 $\mu\text{g}/\text{l}$ produced 100% mortality of eggs and 0.52 $\mu\text{g}/\text{l}$ resulted in 59-67% hatchability, compared to 71-76% for control populations (Nebeker, et al., 1974). Other animal species tested in the present study were somewhat more resistant to Aroclor 1254, requiring 100 $\mu\text{g}/\text{l}$ for complete lethality of embryo-larval stages. Probit LC_{50} values were 0.4, 0.7, 3.5, 5.2, 6.4, 10.3, and 38.2 $\mu\text{g}/\text{l}$ for rainbow trout, redear sunfish, leopard frog, channel catfish, goldfish, American toad, and Fowler's toad at hatching, and 0.3, 0.5, 1.0, 1.8, 1.2, 2.0, and 3.7 $\mu\text{g}/\text{l}$ at 4 days posthatching (Table 4). LC_1 's calculated at 4 days posthatching varied from 0.01 $\mu\text{g}/\text{l}$ for trout to 0.07 $\mu\text{g}/\text{l}$ for Fowler's toad (Table 6). While posthatched mortality was inconsequential for trout and sunfish, larval survival for both species of toad was greatly reduced.

In tests with Aroclor 1242, trout again was the most sensitive species, suffering a 12% reduction in hatchability at 0.1 $\mu\text{g}/\text{l}$ and complete lethality at 100 $\mu\text{g}/\text{l}$. Hatched and posthatched LC_{50} 's in $\mu\text{g}/\text{l}$ were 1.3 and 1.0 for trout, 4.7 and 3.6 for sunfish, 7.2 and 4.2 for catfish, 7.8 and 2.1 for the leopard frog, 13.1 and 2.6 for goldfish, 50.2 and 2.7 for the American toad, and 131.1 and 12.1 for Fowler's toad (Table 4). At hatching, these LC_{50} 's reflect differences in species sensitivity of up to two orders of magnitude. The LC_1 's calculated at 4 days posthatching varied from 0.01 $\mu\text{g}/\text{l}$ for trout to 0.22 $\mu\text{g}/\text{l}$ for Fowler's toad (Table 6). As in bioassays with Aroclor 1254, toad embryos were relatively resistant to Aroclor 1242, but survival frequencies of 4-day larvae dropped sharply. All test species were somewhat more tolerant to Aroclor 1242 than Aroclor 1254. This finding is in agreement with previous studies on newly hatched fry of the fathead minnow, in which 96-hour LC_{50} 's were found to be 7.7 and 15.0 $\mu\text{g}/\text{l}$ for Aroclor 1254 and Aroclor 1242, respectively (Nebeker, et al., 1974).

The least toxic of the four PCB's were Aroclor 1016 and Capacitor 21 (Table 2). Depending on the animal species tested, concentrations of 100 to 1000 $\mu\text{g}/\text{l}$ were required to produce complete lethality of embryo-larval stages. In tests with Capacitor 21, median lethal concentrations at 4 days posthatching were 1.6, 2.9, 5.2, 6.5, 10.0, 14.9, and 28.0 $\mu\text{g}/\text{l}$ using trout, leopard frog, catfish, redear sunfish, American toad, goldfish, and Fowler's toad, respectively (Table 5). Given in the same order, LC_{50} 's for Aroclor 1016 were 1.1, 6.2, 11.2, 7.8, 7.2, 13.2, and 27.7 $\mu\text{g}/\text{l}$. Both compounds produced significant levels of posthatched mortality for all species, except trout and sunfish.

Based on LC_{50} values calculated for the PCB compounds at 4 days posthatching, trout was invariably the most sensitive and Fowler's toad the most tolerant species. A similar trend has been observed previously in bioassays with several inorganic toxicants, such as boron (Birge and Black, 1977a) and copper (Birge and Black, 1979). Although the order of sensitivity for the remaining species varied somewhat with the Aroclor compound administered, the range of LC_{50} 's at 4 days posthatching was surprisingly narrow (i.e., 0.5-14.9 $\mu\text{g}/\text{l}$). Except for Aroclor 1016 and

Capacitor 21, which produced similar responses, the toxicity of the PCB's increased with percent chlorine composition, which was 21%, 41%, 42%, and 54% for Capacitor 21, Aroclors 1016, 1242, and 1254, respectively. In most instances, Aroclor 1254 gave LC_{50} and LC_1 values 5-10 times lower than those for Aroclor 1016 and Capacitor 21. Similarly, Hansen, et al. (1975) found that fry of the sheepshead minnow were substantially more sensitive to Aroclor 1254 than to Aroclor 1016.

Embryos and early larvae appear to be far less tolerant to PCB's than are adult fish. In intermittent flow tests with adult channel catfish, Stalling and Mayer (1972) reported 15-day, 20-day, and 25-day LC_{50} 's of 741, 300, and 113 $\mu\text{g}/\text{l}$ for Aroclor 1254. With rainbow trout this compound gave 5-day and 10-day LC_{50} 's of 156 and 8 $\mu\text{g}/\text{l}$. These values are 25 to 490 times greater than the LC_{50} 's calculated for embryo-larval stages of the same two species (Table 4).

The debilitating effects of PCB's on development were not limited to embryonic and larval mortality, as the four compounds produced significant numbers of teratic larvae. In all instances, the occurrence of embryonic anomalies ranged from 0-3% at near-threshold concentrations and increased proportionately with toxicant concentration, reaching frequencies as high as 60% and 100% in tests with the goldfish and American toad (Table 2). However, considering overall effects, embryonic mortality was a more sensitive test response than teratogenesis. Teratic defects observed in experimental populations did not differ substantially from those previously described for boron and certain heavy metals (Birge and Black, 1977a, b). The skeletal system was the most sensitive indicator of teratogenic responses in fish, and the most common defects included moderate to acute lordosis, scoliosis, kyphosis, and rigid coiling of the vertebral columns. In addition, there were infrequent occurrences of dwarfed bodies, defective fins, and absent or reduced eyes. Anomalies observed among amphibian populations were about evenly divided between skeletal defects and acute abdominal edema.

Additional static renewal bioassays were conducted on catfish, goldfish, leopard frog, and Fowler's toad with three chemicals proposed as PCB replacement products. Each compound was administered at five

concentrations ranging from 0.01 to 100 mg/l, and survival data are presented in Table 3. Probit LC_{50} and LC_1 values, computed by counting grossly anomalous survivors as lethals, are given in Tables 7 and 8. The two phthalate compounds (i.e., DOP, DINP) showed similar toxicity to each of the test species. Catfish embryos were the most sensitive, suffering 8-10% mortality at 0.1 mg/l and complete lethality at 10.0 mg/l (Table 3). At 4 days posthatching, LC_1 's and LC_{50} 's were 0.01 and 0.42 mg/l for DINP and 0.03 and 0.69 mg/l for DOP. The LC_{50} values calculated for the remaining species varied from 4.9-77.2 mg/l at hatching, giving a decreasing order of sensitivity of leopard frog, Fowler's toad, and redear sunfish (Tables 7, 8). At 4 days, the LC_{50} range decreased to 3.0-6.2 mg/l. Appreciable levels of posthatched mortality were observed for the sunfish and Fowler's toad.

Dow Corning 561 silicone, a polydimethylsiloxane (PDMS), proved the least toxic of all compounds tested, as significant reductions in survival did not occur until exposure concentrations reached 1-10 mg/l (Table 3). Except for the sunfish, complete lethality of posthatched larvae was achieved at 100 mg/l. The LC_{50} 's at hatching were 5.6, 17.6, 134.8, and 307.1 mg/l for catfish, leopard frog, Fowler's toad, and sunfish, respectively, and at 4 days posthatching these values decreased to 3.2, 7.0, 7.7, and 37.8 mg/l. Probit LC_1 's ranged from 0.04-0.30 mg/l at 4 days posthatching. Conducting static bioassays, Hobbs, et al. (1975) found other PDMS materials to be relatively non-toxic to several aquatic animals. For example, the 48-hour TL_{50} for Dow Corning 200 fluid was 44.5 mg/l in tests with Daphnia magna, and 96-hour TL_{50} 's exceeded 10,000 mg/l when rainbow trout and bluegill sunfish were exposed to Dow Corning Antifoam C emulsion.

Frequencies of teratic larvae produced by the three replacement compounds were highest for catfish and sunfish, and lowest among amphibian populations. While the types of embryonic anomalies were similar to those produced by the PCB's, occurrences of teratogenesis were much less prevalent with DOP, DINP, and DC 561. In addition to producing fewer anomalous organisms, the proposed replacement products proved far less lethal to embryo-larval stages than did any of the PCB's.

For the test species exposed to all seven chemicals, LC_{50} 's at both hatching and 4 days posthatching reflected a difference in effect of 2-5 orders of magnitude between the PCB and replacement compounds. For example, in bioassays with the redear sunfish, LC_{50} 's at hatching were $0.69 \mu\text{g/l}$ for Aroclor 1254 and 307 mg/l for DC 561. When calculated as a potency ratio (*i.e.*, $\frac{307 \text{ mg/l}}{0.69 \mu\text{g/l}}$), the differential toxicity between these two compounds exceeded 440,000.

Flow-through bioassays. Continuous flow bioassays were conducted with Capacitor 21 and DOP on embryo-larval stages of fish, using two levels of water hardness (*i.e.*, 50 and 200 mg/l CaCO_3). As described previously, the flow-through system (Figure 1) was designed to test insoluble and volatile organics without the use of carrier solvents. Survival, teratogenesis, and toxicant monitoring data are given in Tables 9 and 10. The LC_{50} and LC_1 values given in Tables 11 and 12 were calculated to include grossly anomalous organisms as lethals.

In tests with Capacitor 21, bass larvae suffered nearly complete mortality at concentrations of 2.4-2.9 $\mu\text{g/l}$, and survival was impaired by 10% at exposures as low as 0.4 $\mu\text{g/l}$. Depending on water hardness, LC_{50} 's ranged from 2.3-2.7 $\mu\text{g/l}$ at hatching and from 1.5-1.8 $\mu\text{g/l}$ at 4 days posthatching (Table 11). Probit LC_1 values at 4 days varied from 0.5-0.9 $\mu\text{g/l}$ (Table 12). Developmental stages of the redear sunfish were somewhat more tolerant than bass to Capacitor 21, and this is reflected in the 4 day LC_{50} 's of 8.5-13.5 $\mu\text{g/l}$. These values were in very close agreement with the Capacitor 21 LC_{50} of 6.5 $\mu\text{g/l}$ determined for sunfish in static renewal tests (Table 5). Occurrences of teratogenesis were found to be concentration dependent and reached 9% and 12% in hatched populations of bass and sunfish, respectively.

DOP was administered to eggs and larvae of the largemouth bass at toxicant concentrations of 0.06-149 mg/l , and probit LC_{50} 's ranged from 63.9-66.1 mg/l at hatching and 32.9-42.1 mg/l at 4 days posthatching. The LC_1 values at 4 days varied from 7.2-7.3 $\mu\text{g/l}$. Trout developmental stages exhibited a greater tolerance than bass to DOP, as LC_{50} 's were 139.1-154.0 mg/l at hatching and 139.5-149.2 mg/l at 4 days posthatching. Moreover, LC_1 's calculated for trout ranged from 150-680 $\mu\text{g/l}$, values

approximately 20-90 times those determined for bass. For both piscine species, teratic embryos were observed infrequently and posthatched mortality was not extreme. Variations in water hardness had no significant effect on the toxicity of either DOP or Capacitor 21 ($P > 0.05$, t test).

Using posthatched LC_{50} 's as a basis for comparison, Capacitor 21 was found to be up to 28,000 times more toxic than DOP to bass. Paralleling results from static renewal bioassays, flow data reflected vast differences in toxicity between PCB compounds and the proposed PCB-replacement products. The good data correlation observed between static renewal procedures and the more intricate and time-consuming flow bioassays points to the special applicability of the former for screening aquatic contaminants. Static renewal embryo-larval tests can serve as a relatively economical and straightforward means to survey many types of environmental contaminants for toxicological properties. Had these methods been employed to examine the toxic effects of polychlorinated biphenyls in advance of their widespread production and use, subsequent environmental problems could have been averted.

CHAPTER IV

CONCLUSIONS

Static renewal tests were conducted on seven species of fish and amphibians with four polychlorinated biphenyl compounds, including Aroclors 1254, 1242, 1016, and Capacitor 21. Depending on the PCB compound administered, the ranges of LC_{50} 's at 4 days posthatching were 0.3-1.6 $\mu\text{g}/\text{l}$ for rainbow trout, 0.5-7.8 $\mu\text{g}/\text{l}$ for redear sunfish, 1.0-6.2 $\mu\text{g}/\text{l}$ for leopard frog, 1.2-14.9 $\mu\text{g}/\text{l}$ for goldfish, 1.8-11.2 $\mu\text{g}/\text{l}$ for channel catfish, 2.0-10.0 $\mu\text{g}/\text{l}$ for American toad, and 3.7-28.0 $\mu\text{g}/\text{l}$ for Fowler's toad. The LC_1 's ranged from 0.009-0.011 $\mu\text{g}/\text{l}$ for trout, 0.02-0.26 $\mu\text{g}/\text{l}$ for redear sunfish, 0.02-0.10 $\mu\text{g}/\text{l}$ for leopard frog, 0.02-0.20 $\mu\text{g}/\text{l}$ for goldfish, 0.05-0.18 $\mu\text{g}/\text{l}$ for catfish, 0.02-0.35 $\mu\text{g}/\text{l}$ for American toad, and 0.07-0.55 $\mu\text{g}/\text{l}$ for Fowler's toad. Concentrations of 10-1000 $\mu\text{g}/\text{l}$ produced complete lethality of embryo-larval stages. The toxicity of the PCB's generally increased in the order of increasing chlorine composition. Rainbow trout was found to be the most susceptible test animal and Fowler's toad the most tolerant. Although embryonic mortality was the most sensitive test response for all species, posthatched lethality was substantial for Fowler's toad, American toad, and goldfish. In addition, teratogenesis occurred frequently in nearly all tests and was observed to be concentration dependent.

Three proposed PCB-replacement products, including DOP, DINP, and DC 561 proved far less toxic than PCB's to developmental stages. At 4 days posthatching, the ranges of LC_{50} 's were 0.4-3.2 mg/l for catfish, 3.0-7.7 mg/l for Fowler's toad, 3.6-7.0 mg/l for leopard frog, and 4.7-37.8 mg/l for redear sunfish. Probit LC_1 's varied from 0.01-0.04, 0.03-0.25, 0.11-0.26, and 0.14-0.30 mg/l for catfish, Fowler's toad, redear sunfish, and leopard frog, respectively. Concentrations as high as 100 mg/l generally were required to produce complete lethality. The decreasing order of toxicity was found to be DINP, DOP, and DC 561, and

the LC_{50} 's for these compounds were 2-5 orders of magnitude higher than those for polychlorinated biphenyls. Moreover, occurrences of teratic larvae were much less frequent with the proposed replacement products.

Using a flow-through system designed to test volatile and insoluble organics without the use of carrier solvents, additional embryo-larval bioassays were conducted with Capacitor 21 and DOP at two levels of water hardness (*i.e.*, 50 and 200 mg/l $CaCO_3$). At 4 days posthatching, the ranges of LC_1 's for Capacitor 21 were 0.5-0.9 and 1.3-3.5 $\mu\text{g/l}$ in tests with the largemouth bass and redear sunfish, respectively. Median lethal concentrations calculated for these two species varied from 1.5-1.8 $\mu\text{g/l}$ and 8.5-13.5 $\mu\text{g/l}$, respectively. The latter values are very similar to the LC_{50} determined in static renewal tests with the redear sunfish, indicating good correlation between the two bioassay methods. Corresponding to results from static renewal tests, DOP was far less toxic than Capacitor 21. At 4 days posthatching, LC_{50} values for DOP ranged from 32.9-42.1 mg/l for bass and 139.5-149.2 mg/l for trout. Teratogenesis was low for both species, and posthatched mortality was not severe. Variations in water hardness did not exert a significant effect on the toxicity of either compound ($P > 0.05$).

As has been observed for a considerable number of other aquatic contaminants (Birge and Black, 1979; Birge, *et al.*, 1979a, b), PCB's were appreciably more toxic to developmental stages than to adult organisms. Because of their high sensitivity, embryo-larval tests should prove useful in establishing protective guidelines for aquatic ecosystems. For example, the limiting LC_1 values calculated for PCB's (*e.g.*, Aroclor 1254 to trout) closely approached the EPA freshwater criterion of 0.001 $\mu\text{g/l}$. Recommended environmental levels have not as yet been established for DOP, DINP, and DC 561. However, the findings obtained in this study indicate that these chemicals may be far less hazardous to aquatic biota than are polychlorinated biphenyl compounds.

Table 2. Effects of PCB compounds on embryo-larval stages of fish and amphibians.

Species	PCB Concentration ($\mu\text{g}/\text{l}$)	Percent Survival at Hatching (H) and 4 Days Posthatching (PH) ¹							
		Aroclor 1254		Aroclor 1242		Aroclor 1016		Capacitor 21	
		H	PH	H	PH	H	PH	H	PH
American Toad	0.1	96(2)	93	97(1)	94	100	99	100	99
	1.0	82(5)	70	89(6)	73	97(1)	91	95	90
	10.0	68(13)	33	71(10)	41	85(16)	59	81(12)	61
	100.0	39(25)	0	57(32)	0	66(29)	12	68(21)	23
	1000.0	14(100)	0	51(47)	0	63(34)	0	64(31)	0
19 Channel Catfish	0.1	97(1)	95	100	100	102(1)	100	101	99
	1.0	82(7)	66	88(2)	81	98(2)	94	91(3)	86
	10.0	49(5)	18	53(5)	37	83(6)	63	60(2)	41
	100.0	0	0	0	0	45(20)	0	0	0
	1000.0	0	0	0	0	0	0	0	0
Fowler's Toad	0.1	99(1)	95	100	99	100(1)	99	100	99
	1.0	94(2)	79	98(1)	92	98	93	99	96
	10.0	77(4)	38	83(2)	57	92(1)	64	90(1)	72
	100.0	47(9)	0	65(6)	15	84(2)	40	66(6)	31
	1000.0	0	0	21(13)	0	37(11)	0	28(10)	0
Goldfish	0.1	95	92	98	97	98	99	99	100
	1.0	78(10)	60	79(1)	68	96	92	89(1)	86
	10.0	57(20)	26	66(16)	41	82(10)	73	83(6)	71
	100.0	28(27)	0	34(39)	0	57(18)	0	61(13)	20
	1000.0	0	0	24(60)	0	32(30)	0	21(39)	0

Table 2 - continued.

Species	PCB Concentration ($\mu\text{g}/\text{l}$)	Percent Survival at Hatching (H) and 4 Days Posthatching (PH) ¹							
		Aroclor 1254		Aroclor 1242		Aroclor 1016		Capacitor 21	
		H	PH	H	PH	H	PH	H	PH
Leopard Frog	0.1	93	90	98	96	98	97	98	96
	1.0	74(2)	56	80(2)	68	86	79	80(1)	67
	10.0	48(18)	17	61(5)	25	72	56	64(3)	37
	100.0	0	0	14(55)	0	37	0	16(41)	0
	1000.0	0	0	0	0	0	0	0	0
20 Rainbow Trout	0.1	80(4)	78	88	87	87	87	85	84
	1.0	31(4)	28	57(9)	54	62(15)	60	59(4)	54
	10.0	0	0	22(5)	17	18(6)	17	38(8)	35
	100.0	0	0	0	0	0	0	0	0
	1000.0	0	0	0	0	0	0	0	0
Redear Sunfish	0.1	84	83	97	97	99(1)	99	98	98
	1.0	58(8)	44	87(2)	85	87	87	96(2)	87
	10.0	0	0	46(27)	34	69(3)	46	53(15)	45
	100.0	0	0	0	0	50(16)	12	0	0
	1000.0	0	0	0	0	0	0	0	0

¹Frequencies of teratic larvae expressed parenthetically.

Table 3. Effects of proposed PCB-replacement compounds on embryo-larval stages of fish and amphibians.

Species	Concentration (mg/l)	Percent Survival at Hatching (H) and 4 Days Posthatching (PH) ¹					
		DINP		DOP		DC 561	
		H	PH	H	PH	H	PH
Channel Catfish	0.01	99	98	101(1)	100	101	100
	0.10	90(2)	82	92(3)	90	97(1)	96
	1.00	64(13)	40	75(13)	55	81(4)	73
	10.00	0	0	0	0	54(5)	38
	100.00	0	0	0	0	0	0
Fowler's Toad	0.01	100	99	100	100	100	100
	0.10	97	93	97	95	99	99
	1.00	84(1)	70	86	75	92	86
	10.00	68	35	70	39	82(1)	55
	100.00	28(5)	0	40(1)	0	52	0
Leopard Frog	0.01	100	100	101	100	101(1)	100
	0.10	100(1)	99	100	100	99	99
	1.00	87	82	88(1)	84	92	89
	10.00	35(4)	27	39(5)	34	54	47
	100.00	0	0	0	0	25(2)	0
Redear Sunfish	0.01	100	100	100	100	100	100
	0.10	99	98	99	100	100	100
	1.00	94(2)	80	94(1)	88	99	95
	10.00	79(9)	47	80(9)	51	92(4)	77
	100.00	60(18)	0	60(18)	0	78(10)	41

¹Frequencies of teratic larvae expressed parenthetically.

Table 4. Log probit analysis of LC₅₀ values for Aroclor 1254 and Aroclor 1242.

Species	Exposure Days Beyond Hatching	Aroclor 1254		Aroclor 1242	
		LC ₅₀ (µg/l)	95% Confidence Limits	LC ₅₀ (µg/l)	95% Confidence Limits
American Toad	0	10.32	6.67 - 15.79	50.17	29.92 - 87.49
	4	2.02	1.44 - 2.77	2.71	1.91 - 3.75
Channel Catfish	0	5.22	4.03 - 6.65	7.20	5.68 - 9.01
	4	1.76	1.36 - 2.24	4.24	3.32 - 5.34
Fowler's Toad	0	38.18	30.21 - 48.06	131.10	100.58 - 173.14
	4	3.74	2.98 - 4.64	12.09	9.74 - 14.91
Goldfish	0	6.35	4.26 - 9.31	13.08	8.70 - 19.53
	4	1.18	0.84 - 1.61	2.64	1.89 - 3.61
Leopard Frog	0	3.51	2.79 - 4.39	7.81	6.22 - 9.76
	4	1.03	0.83 - 1.27	2.13	1.72 - 2.63
Rainbow Trout	0	0.35	0.24 - 0.48	1.26	0.81 - 1.85
	4	0.32	0.22 - 0.45	1.03	0.67 - 1.51
Redear Sunfish	0	0.69	0.52 - 0.90	4.74	3.58 - 6.18
	4	0.53	0.39 - 0.70	3.56	0.65 - 4.66

Table 5. Log probit analysis of LC₅₀ values for Aroclor 1016 and Capacitor 21.

Species	Exposure Days Beyond Hatching	Aroclor 1016		Capacitor 21	
		LC ₅₀ (µg/l)	95% Confidence Limits	LC ₅₀ (µg/l)	95% Confidence Limits
American Toad	0	182.24	108.10 - 333.96	249.85	134.03 - 543.10
	4	7.16	5.39 - 9.34	9.97	7.21 - 13.53
Channel Catfish	0	52.14	40.33 - 66.34	9.26	7.24 - 11.61
	4	11.16	9.93 - 12.97	5.20	4.02 - 6.63
Fowler's Toad	0	471.56	355.92 - 651.80	181.86	138.32 - 243.82
	4	27.72	21.77 - 35.08	28.02	22.59 - 34.47
Goldfish	0	89.08	58.16 - 139.34	69.71	46.19 - 107.05
	4	13.21	10.63 - 16.43	14.89	10.66 - 20.38
Leopard Frog	0	23.32	18.18 - 29.78	9.37	7.40 - 11.78
	4	6.19	4.95 - 7.69	2.87	2.29 - 3.57
Rainbow Trout	0	1.16	0.75 - 1.69	1.83	1.14 - 2.79
	4	1.08	0.70 - 1.56	1.55	0.95 - 2.37
Redear Sunfish	0	32.37	23.01 - 42.22	6.35	4.84 - 8.23
	4	7.82	5.74 - 10.35	6.50	4.81 - 8.56

Table 6. Log probit analysis of LC₁ values at 4 days posthatching for PCB compounds.

Compound	Species	LC ₁ (µg/l)	95% Confidence Limits
Aroclor 1254	American Toad	0.02	0.01 - 0.05
	Channel Catfish	0.05	0.02 - 0.09
	Fowler's Toad	0.07	0.04 - 0.11
	Goldfish	0.02	0.01 - 0.04
	Leopard Frog	0.02	0.01 - 0.03
	Rainbow Trout	0.009	0.003 - 0.020
	Redear Sunfish	0.02	0.01 - 0.04
Aroclor 1242	American Toad	0.03	0.01 - 0.06
	Channel Catfish	0.14	0.07 - 0.23
	Fowler's Toad	0.22	0.12 - 0.36
	Goldfish	0.04	0.01 - 0.08
	Leopard Frog	0.04	0.02 - 0.06
	Rainbow Trout	0.010	0.002 - 0.025
	Redear Sunfish	0.19	0.08 - 0.35
Aroclor 1016	American Toad	0.35	0.15 - 0.64
	Channel Catfish	0.08	- -
	Fowler's Toad	0.18	0.09 - 0.33
	Goldfish	0.10	- -
	Leopard Frog	0.10	0.05 - 0.16
	Rainbow Trout	0.011	0.003 - 0.027
	Redear Sunfish	0.26	0.10 - 0.51
Capacitor 21	American Toad	0.21	0.08 - 0.42
	Channel Catfish	0.18	0.08 - 0.31
	Fowler's Toad	0.55	0.30 - 0.91
	Goldfish	0.20	0.07 - 0.42
	Leopard Frog	0.03	0.02 - 0.06
	Rainbow Trout	0.010	0.001 - 0.017
	Redear Sunfish	0.25	0.10 - 0.48

Table 7. Log probit analysis of LC₅₀ values for proposed PCB-replacement compounds.

Species	Exposure Days Beyond Hatching	DINP		DOP		DC 561	
		LC ₅₀ (mg/l)	95% Confidence Limits	LC ₅₀ (mg/l)	95% Confidence Limits	LC ₅₀ (mg/l)	95% Confidence Limits
Channel Catfish	0	0.87	0.68 - 1.08	1.21	1.11 - 1.46	5.57	4.23 - 7.21
	4	0.42	0.33 - 0.53	0.69	0.55 - 0.86	3.16	2.36 - 4.15
Fowler's Toad	0	23.51	17.15 - 33.42	44.14	30.33 - 68.84	134.76	83.97 - 253.01
	4	2.95	2.32 - 3.73	3.88	3.08 - 4.84	7.68	6.24 - 9.37
Leopard Frog	0	4.94	4.08 - 5.95	5.52	4.56 - 6.65	17.55	13.57 - 22.96
	4	3.63	2.97 - 4.40	4.44	3.65 - 5.37	6.95	5.73 - 8.40
Redear Sunfish	0	71.85	45.11 - 129.71	77.20	47.78 - 142.99	307.11	163.26 - 852.16
	4	4.67	3.46 - 6.16	6.18	4.65 - 8.04	37.79	26.27 - 56.73

Table 8. Log probit analysis of LC₁ values at 4 days posthatching for proposed PCB-replacement compounds.

Compound	Species	LC ₁ (mg/l)	95% Confidence Limits
DINP	Channel Catfish	0.01	0.01 - 0.02
	Fowler's Toad	0.03	0.01 - 0.05
	Leopard Frog	0.14	0.08 - 0.22
	Redear Sunfish	0.11	0.04 - 0.22
DOP	Channel Catfish	0.03	0.02 - 0.05
	Fowler's Toad	0.06	0.03 - 0.10
	Leopard Frog	0.18	0.10 - 0.27
	Redear Sunfish	0.26	0.11 - 0.46
DC 561	Channel Catfish	0.04	0.02 - 0.09
	Fowler's Toad	0.25	0.14 - 0.40
	Leopard Frog	0.30	0.17 - 0.46
	Redear Sunfish	0.23	0.05 - 0.62

Table 9. Effects of Capacitor 21 on embryo-larval stages of fish exposed in a flow-through system.

Species	Water Hardness (mg/l CaCO ₃)	Capacitor 21 Concentration Mean ± S.E. (µg/l)	Percent Survival	
			Hatching ¹	4 Days Posthatching
Largemouth Bass	50	0.5 ± 0.2	98	93
		0.8 ± 0.3	93(1)	86
		1.5 ± 0.5	82(2)	66
		2.4 ± 0.9	48(8)	10
	200	0.4 ± 0.1	97	90
		0.5 ± 0.1	98(1)	94
		1.5 ± 0.2	85(1)	69
		2.9 ± 0.7	47(9)	11
Redear Sunfish	50	1.7 ± 0.5	96	94
		2.1 ± 0.4	96(1)	94
		6.5 ± 2.7	94	86
		17.1 ± 3.8	63(12)	40
	200	1.4 ± 0.3	98	95
		2.2 ± 0.7	96	91
		4.5 ± 1.7	91(5)	86
		11.0 ± 1.4	64(9)	38

¹Frequencies of teratic larvae expressed parenthetically.

Table 10. Effects of dioctylphthalate (DOP) on embryo-larval stages of fish exposed in a flow-through system.

Species	Water Hardness (mg/l CaCO ₃)	DOP Concentration Mean ± S.E. (mg/l)	Percent Survival	
			Hatching ¹	4 Days Posthatching
Largemouth Bass	50	0.055 ± 0.006	97	95
		0.30 ± 0.03	93	91
		46.33 ± 3.95	74	67
		66.88 ± 3.31	39(1)	26
		149.0 ± 14.7	13(4)	2
	200	0.065 ± 0.012	97	96
		0.30 ± 0.03	91(1)	90
		35.50 ± 3.10	71	64
		60.57 ± 4.29	39(1)	30
		146.0 ± 16.3	16(3)	7
Rainbow Trout	50	0.040 ± 0.004	100	100
		0.101 ± 0.008	99(1)	100
		0.476 ± 0.033	95	95
		55.34 ± 3.09	94(3)	94
		71.87 ± 3.21	89(5)	87
	148.2 ± 9.5	53(16)	51	
	200	0.045 ± 0.006	99(1)	96
		0.102 ± 0.007	97(1)	97
		0.501 ± 0.027	92(2)	91
		48.91 ± 3.98	87(3)	86
88.80 ± 7.99		82(7)	78	
141.5 ± 11.6	54(12)	50		

¹Frequencies of teratic larvae expressed parenthetically.

Table 11. Log probit analysis of LC₅₀ values for dioctylphthalate (DOP) and Capacitor 21 administered in a flow-through system.

Compound	Species	Exposure Days Beyond Hatching	50 mg/l CaCO ₃		200 mg/l CaCO ₃	
			LC ₅₀ (mg/l)	95% Confidence Limits	LC ₅₀ (mg/l)	95% Confidence Limits
DOP	Largemouth Bass	0	63.9	58.3 - 69.7	66.1	36.7 - 140.1
		4	42.1	24.8 - 77.8	32.9	19.8 - 58.9
	Rainbow Trout	0	139.1	122.8 - 164.9	154.0	127.0 - 216.0
		4	139.5	123.2 - 165.2	149.2	125.8 - 203.8
Capacitor 21	Largemouth Bass	0	0.0023	0.0021 - 0.0027	0.0027	0.0024 - 0.0030
		4	0.0015	0.0014 - 0.0016	0.0018	0.0017 - 0.0019
	Redear Sunfish	0	0.0191	0.0156 - 0.0288	0.0137	0.0105 - 0.0802
		4	0.0135	0.0113 - 0.0165	0.0085	0.0071 - 0.0108

Table 12. Log probit analysis of LC₁ values at 4 days posthatching for dioctylphthalate (DOP) and Capacitor 21 administered in a flow-through system.

Compound	Species	50 mg/l CaCO ₃		200 mg/l CaCO ₃	
		LC ₁ (µg/l)	95% Confidence Limits	LC ₁ (µg/l)	95% Confidence Limits
DOP	Largemouth Bass	7.2	0.9 - 29.4	7.3	0.9 - 28.6
	Rainbow Trout	150.0	- -	680.0	- -
Capacitor 21	Largemouth Bass	0.5	0.4 - 0.7	0.9	0.7 - 1.0
	Redear Sunfish	3.5	1.6 - 5.1	1.3	0.6 - 2.0

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