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TOXICITY OF POLYCHLORINATED BIPHENYLS (PCB's) TO FISH AND OTHER AQUATIC LIFE

by

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SUMMARY OF FINDINGS (ABSTRACT)

Aroclor 1248 was the most toxic of the eight aroclors tested to Daphnia magna in static tests, with a 3-week LC50 of 25 µg/liter. Aroclor 1254 was most toxic under continuous-flow conditions with a 3-week LC50 of 1.3 µg/liter. The Aroclors were much more toxic under continuous-flow than static conditions, with 16 percent impairment of reproduction by Aroclor 1248 occurring at 1.0 µg/liter.

Calculated 96-hour LC50 values for newly-hatched fathead minnows were 7.7 µg/l for Aroclor 1254 and 15 µg/l for 1242. Three-month-old fatheads had a 96-hour LC50 of ca. 300 µg/l for 1242. Reproduction occurred at and below 1.8 µg/l 1254 and at and below 5.4 µg/l 1242. Polychlorinated biphenyls were acutely toxic but exhibited much greater chronic toxicity at very low levels, due to their cumulative nature. Newly hatched young were the most sensitive life stage. Young fathead growth was also affected above 2.2 µg/l 1248 and none survived above 5.1 µg/l after 30 days. Young flagfish, Jordanella floridae, did not survive above 5.1 µg/l 1248 and did not grow well above 2.2 µg/l.

Ninety-six hour LC50 values for Aroclor 1242 and 1248 with Gammarus pseudolimnaeus were 73 and 29 µg/liter. Survival after 30 days was 53 percent at 8.7 µg/l 1242 and 52 percent at 5.1 µg/l 1248. Good reproduction and survival of young occurred at 2.8 µg/l 1242 and 2.2 µg/l 1248.

Abundant adult emergence of the midge Tanytarsus dissimilis did not occur above 5 µg/l 1248 or 3.5 µg/l 1254. The calculated 3-week LC50 (50 percent reduction based on control as 100 percent) for Aroclor 1254 was .65 µg/l for larvae and .45 µg/l for pupae.

Application factors of 0.10 for 1242 and 0.15 for 1248 were calculated for the PCB's and Gammarus. Application factors of 0.2 for 1254 and 0.16 for 1242 were calculated for fathead minnows using newly hatched fish to obtain the 96-hour LC50.

INTRODUCTION

Polychlorinated biphenyls (PCB's) have been shown to be widespread in the environment, rivaling DDT in general occurrence (Risebrough and Bodine, 1970). Their significance in the aquatic environment as a poison is now being revealed. Duke, Lowe, and Wilson (1970) have documented the occurrence of one of the many PCB mixtures (Aroclor 1254) in the water, sediment, and biota of Escambia Bay, Florida. Veith and Lee (1970) reviewed the chlorinated biphenyl contamination in natural waters and stated that the PCB's may be one of the more widespread contaminants. A general review of the structural and physical properties, uses, analytical methods of analysis, levels found in nature, and toxicology of PCB's can be found in the paper by Peakall and Lincer (1970).

Polychlorinated biphenyl compounds are just one of many exotic chemicals now finding their way into our waterways. They are being detected in fish and other aquatic life at levels much higher than concentrations found in the water. Because of the only recent availability of analytical methodology for detecting and identifying low levels of PCB's in water and aquatic organisms little is known about their interactions.

The acute toxicity of some of the many types of PCB's produced commercially (Monsanto Chemical Co.) has been demonstrated for a few species of fish, and fish food organisms, such as shrimp, scuds, and aquatic insects (Zitco, 1970; Stalling, 1970; Wildish, 1970; Saunders, in press; Duke, et al., 1970). Little information is currently available

on the chronic effects of PCB on the full life cycles of aquatic animals. The toxicity of these materials must be known so that proper safeguards can be established for preventing further contamination of our waterways and for protecting the complex biological balance that must be maintained in our streams and lakes.

In order to assess the danger of these compounds to fish and fish food organisms this laboratory designed and conducted bioassays using Daphnia magna, the fathead minnow Pimephales promelas, the flagfish Jordanella floridae, the scud Gammarus pseudolimnaeus, and the midge Tanytarsus dissimilis, using commercially available PCB mixtures (Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268). Seven continuous-flow bioassays using Aroclor 1248 and 1254, twenty-four 3-week static bioassays using all eight Aroclors, and 3 PCB mixture bioassays were conducted with Daphnia magna using survival and reproductive success as the measure of toxicity. Aroclor 1242, 1248, and 1254 were utilized to determine their effect on the survival and growth of young fathead minnows and Jordanella. In depth studies were conducted to determine the effects of the two PCB's 1242 and 1254 on growth and maturation, egg production, egg hatch, and young production and survival of the fathead minnow. This study also determined the effects of Aroclor 1242 and 1248 on the growth, survival and reproduction of Gammarus, and the effects of 1248 and 1254 on the growth, survival, and adult emergence of the midge T. dissimilis.

MONITORING PCB's IN BIOASSAY SYSTEMS

Introduction

Since Jensen (1966) identified Polychlorinated Biphenyls (PCB's) in animal tissue concern has mounted as to their hazard in the environment. Several PCB reviews by Risebrough, et al. (1969), Peakall and Lincer (1970) and Veith and Lee (1970) report PCB's being found in water, sediment, fish, birds, and mammals including humans. Their discussion of physical and chemical properties point out the difficulties present in the available methods of analysis.

The low solubility of PCB compounds in water caused some difficulties when the experiments required high starting concentrations. Zitko (1970) found that by adding a nonionic surfactant to the PCB's he could increase the dosing level in his experiments. We used this method during preliminary experiments with Daphnia but found the toxicant level higher than needed and discontinued using the surfactant in the remaining bioassays.

Reynolds (1969) pointed out that PCB's caused interference with pesticide residue analysis and many residue chemists found that they had been erroneously reporting high levels of chlorinated pesticides because of this. The fact that PCB's can be isolated with chlorinated pesticides gave us a starting point for developing a PCB monitoring procedure. The F. D. A. Pesticide Analytical Manual (1968) has a method for chlorinated pesticide residue analysis that was adaptable to our needs. Briefly, the method calls for an extraction, acetonitrile partitioning, florisil column cleanup and then injection into a gas

chromatograph (G. C.). However, after the Florisil cleanup the PCB's and pesticides have been jointly isolated and need further separation before the G. C. step.

Separation can be achieved by a reaction to alter or destroy the pesticides, leaving the PCB's, or by physically isolating one from the other. The reactions include saponification, reported by Risebrough, et al. (1968), which will dehydrohalogenate many of the pesticides but not PCB's; and a mild nitration also reported by Risebrough, et al. (1969), at 0° C which will also react with pesticides and not PCB's. Reynolds (1969) and Armour and Burke (1969) attempted to repeat the nitration procedure but found complex chromatographs that couldn't be related to the untreated residue.

Physical separation was accomplished by Reynolds (1969) using an activated Florisil column and eluding with n-hexane instead of the n-hexane-ethyl ether used for the pesticide cleanup. Armour and Burke (1970) achieved a similar separation using a silicic acid-celite column and eluding the PCB's with petroleum ether. In both the chemical and physical methods of separation several pesticides are still isolated with the PCB's which have to be corrected for later in the analysis.

Identifying the PCB mixture in the sample is usually done by G. C. using an electron capture detector and comparing the chromatograph with chromatographs of "standard" commercial mixtures.

Quantitative interpretations of the chromatograph are as numerous as there are investigators using them.

Koeman, et al (1969) semiquantitated residues from test animals by using one of the peaks of the "pure" PCB toxicant as an internal standard. Risebrough, et al. (1969), using electron capture, assumed each PCB compound produced the same peak height as an equal amount of pp-DDE. The total of the summed peak heights was multiplied by a correction factor derived from similar measurements of standard solutions using electron capture and microcoulometric detectors. Jensen et al. (1969) estimated total PCB's by summing all PCB components based on combined data from Mass Spectrometry (M. S.), and electron capture and microcoulometric detection. The F. D. A. Pesticide Analytical Manual (1968) has methods for multiple component pesticides such as toxaphene that compares the total of peak heights or areas with the same measurement on a standard of that mixture. A number of other methods include variations or combinations of the above methods but, in all cases there is some question as to how quantitative these results are. Most investigators agree that until the individual compounds are isolated and quantitated with their individual standard any result will be no better than semiquantitative.

METHODS AND MATERIALS

Analytical Methods

Static Daphnia tests. -- Test solutions were prepared by weighing the Aroclor samples using an analytical balance and transferring them to volumetric flasks with acetone and triton x-100, a surfactant (maintained at .03 x PCB level) added to help keep the PCB's in 'solution'. The concentrations of acetone and triton X-100 used were tested previously and found to be non-toxic at the levels used during testing. The Triton-PCB mixtures were diluted to 500 ml with acetone and used as stock solutions. Portions of each stock solution were diluted with acetone to give 5 different working stock solutions for each Aroclor. One ml of each working-stock solution and one ml of food suspension were pipetted into a volumetric flask and diluted to 1000 ml with Lake Superior water. Control solutions were prepared with one ml of acetone and one ml of food suspension in a liter of lake water to keep the acetone concentration constant (1ml/liter) in all test solutions.

Static tests to determine the effect of various PCB mixtures were also conducted with Daphnia magna. A mixed solution containing that concentration of each Aroclor which permitted reproduction, but reduced it be 16 percent, was used for these tests.

Various percentages of the mixture were then tested. The mixture consisted of the following concentrations (in µg/liter): Aroclor 1221 = 87, 1232 = 53, 1242 = 48, 1248 = 16, 1254 = 18, 1260 = 22, 1262 = 24, and 1268 = 162.

Continuous-flow Tests. -- Aroclor 1242, 1248, and 1254, manufactured by Monsanto Chemical Company, were the Polychlorinated Biphenyl mixtures used. The stock solutions were prepared by weighing the PCB mixture and dissolving it in acetone. The toxicant levels were maintained in the flowing system by the Mount diluter. The toxicant was introduced into the diluter system as an acetone solution with a glass syringe mounted in a mechanical injecter.

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

Analysis was done on a Tracor MT-220 gas chromatograph using a six foot 1/4 inch O. D. glass column packed with 3 percent OV-1 on chromosorb W 80/90 mesh. A Coulson Conductivity Detector was used in the reductive mode without a catalyst. Sixty ml/min Helium carrier gas was used with a 10 ml/min Helium purge through the furnace when the column was vented. Forty ml/min of electrolytic hydrogen were used for the reductive pyrolysis. The temperatures of the inlet and all transfer lines were kept at 260° C and the column varied from 195° C to 205° C depending on the PCB mixture being measured. The pyrolysis furnace was kept at 820° C.

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

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

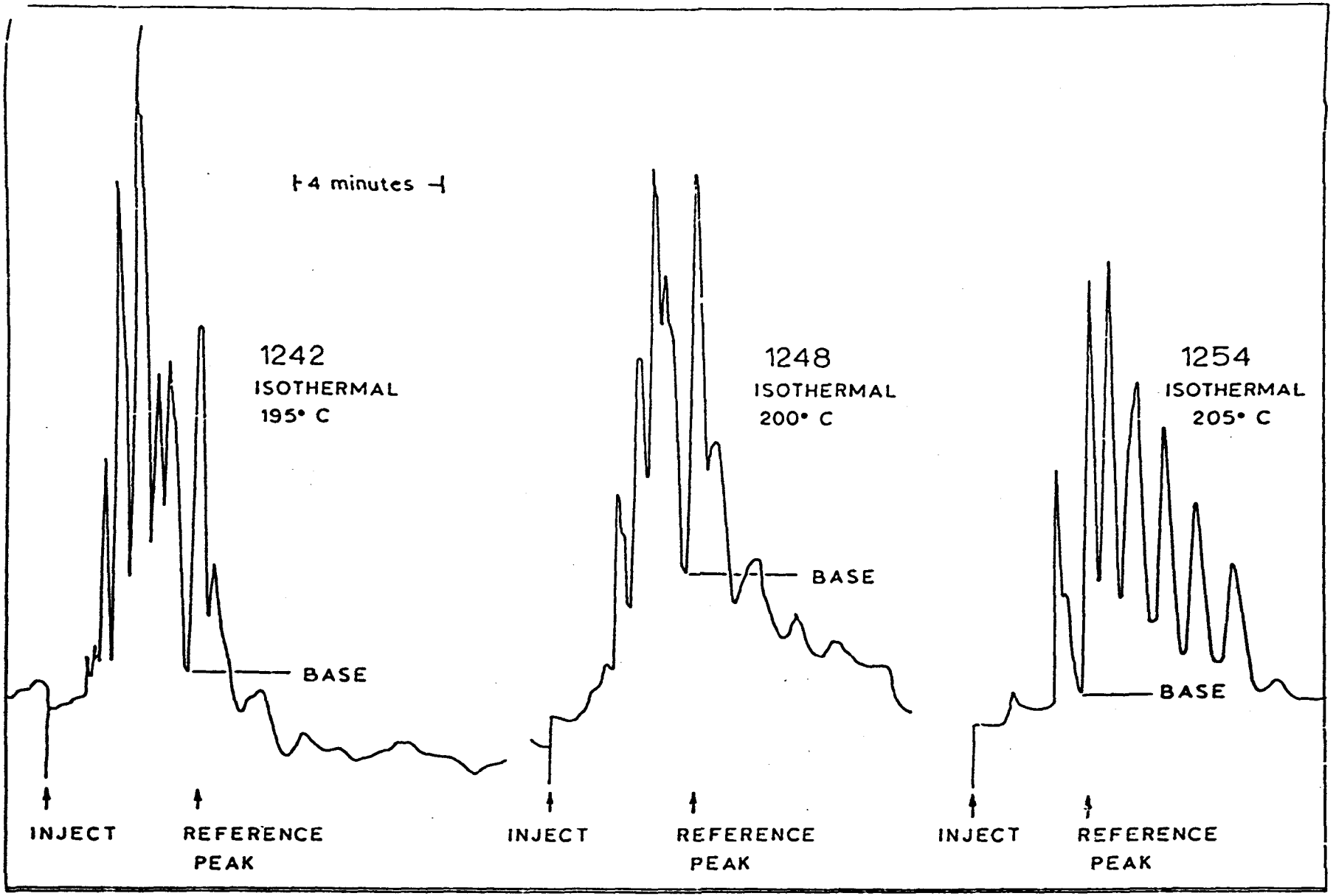

Fig. 1. Single peak used as reference for quantitation of Aroclors 1242, 1248, 1254.

Chemical parameters of the Lake Superior test water that were monitored are listed in Table 1. The weekly measurements were made using the methods from Standard Methods (1965). The dissolved oxygen was measured in the tanks using the azide modification of the winkler method.

Table 1. Chemical Parameters Monitored in Lake Superior Test Water

Parameter	Range
pH	7.5 - 8.0
Acidity	2.4 - 4.0 ppm
Alkalinity	40 - 43 ppm
Calcium hardness	34 - 36 ppm
Total hardness	44 - 46 ppm

Physical Testing Methods

Daphnia Static Tests. -- Test containers were 250 ml glass beakers which were randomly distributed and covered with glass sheets to minimize evaporation. The experiments were conducted at 18 ± 1° C and a photoperiod set for 16 hours light per day.

Continuous-Flow Tests. -- Daphnia were also tested under continuous flow conditions. The fish, Gammarus, and midges were tested only under continuous-flow conditions.

Four complete testing units were utilized, each consisting of five duplicated test concentrations and a control. Stainless steel and glass aquaria were used for all test chambers. Twelve ten-gallon aquaria were used for adult fish in the Aroclor 1254 testing; twelve five-gallon aquaria were utilized for Aroclor 1242 testing; and twenty-four 2 1/2 gallon aquaria were used for testing Daphnia, Gammarus, midges, Jordanella, and some young fathead minnows. Five and 2 1/2 gallon aquaria were used for testing young fish obtained from spawning tanks. Four proportional diluters (Mount and Brungs, 1967) were used to provide the necessary continuous-flow conditions during testing (Figure 2). A modification of the diluter system using a gas-syringe filled with acetone and PCB (no Triton X-100) was utilized with an injector system to deliver the toxicant to the mixing chamber of the diluter (Figure 3). Additional agitation was maintained by circulating the mixing box contents through a submersible pump. Additional mixing boxes, with 2-way and 4-way splits to the duplicate and quadruplicate test aquaria provided further

mixing of the PCB solutions. All tanks were randomly distributed on the test tables. Raw Lake Superior water was utilized for all testing.

The diluters delivered 350 ml (fish tests), 200 ml (fish tests), and 150 ml (Gammarus tests) to each test tank every 3 minutes, with flow rates maintained primarily to ensure adequate dissolved oxygen levels and replenishment of fresh toxic solutions. These flow rates ensured a complete exchange of water in the test tanks about every 4-5 hours. All fish testing tanks were cleaned daily with excess algae or fungus growth scraped away weekly.

Spawning substrates for fathead minnows consisted of inverted longitudinal sections of glass quart beverage bottles, cut to three inches in length. For the hatchability studies the egg cups used to hold eggs until hatching were constructed of 2-inch OD round glass jars with the bottoms cut off. The bottom of the jar was covered with fine stainless steel screen. The cups were hung, partially submersed in the test aquaria and slowly oscillated by means of a rocker arm apparatus (Figure 2) driven by a small electric motor.

Test temperatures were maintained at 24 ± 1° C for the fish tests and 18 ± 1° C for the Daphnia, midge and Gammarus testing. Durotest (Optima FS) and wide spectrum Grow-lux fluorescent tubes provided light for the tests. The photoperiod of Evansville, Indiana, was maintained during testing, with the adjustments in day-length made every two weeks.

Biological Testing Methods

Daphnia static Tests. -- Daphnia magna, obtained from the National Water Quality Laboratory cultures, were tested from young less than 24 hours old, through maturation, reproduction and growth of their young (3 weeks). Five animals were placed in a 250 ml beaker containing 200 ml of test solution with four replicates of each concentration, giving 20 animals at each concentration. The survival of the original animals was recorded and test solutions renewed each week. The young surviving after two weeks were counted and discarded. Young surviving after the third week were also counted and the total young surviving was recorded. All animals were counted and transferred to fresh solutions each week in modified eyedroppers. All tests were repeated three times.

The 3-week LC50, that concentration at which 50 percent of the animals had died after 3 weeks, was used as one measure of toxicity. Fifty percent and sixteen percent reproductive impairment were also used as a measure of reproductive success (Biesinger and Christensen, 1972). This is defined as survival of young as a percentage of the controls or of that test chamber which had the highest number of young produced. The LC50, 50 percent and 16 percent reproductive impairment figures, and 95 percent confident limits for each, were determined using the statistical method of Litchfield and Wilcoxon (1949). The terminology recommended by Sprague (1969) was used to present toxicity data.

Daphnia Static Mixture Tests. -- Various percentages of the PCB mixtures were tested to determine what type of survival would occur if all the PCB's were in an aquatic system at these low levels which do permit reproduction. Testing was conducted as with previous *Daphnia* statics and 50 percent survival and 50 percent and 16 percent reproductive impairment were also calculated as done previously.

Food for the *Daphnia* was prepared by mixing 0.5 grams powdered dried wheat leaves and 10 grams enriched trout food (fry granules) in a blender with 300 ml of Lake Superior water (Biesinger and Christensen, 1972).

Daphnia Continuous-Flow Tests. -- Five animals, less than 24 hours old, were placed in two-gallon (7.6 liter) aquaria, with four replicates of each concentration, giving 20 animals at each concentration. All surviving original *Daphnia*, and their young, were counted at the end of each 2 or 3 week test. Analysis of data and physical-chemical conditions were the same as that for the static tests; however, the animals were fed finely powdered fish food twice a day rather than the solution given to the animals in static tests. Measured PCB concentrations from the continuous-flow tests were used for all calculations.

Fathead Minnow and *Jordanella floridae*. -- All fish were obtained from stocks at the National Water Quality Laboratory, in Duluth, MN. Ninety-six hour acute tests with various ages of fish were conducted according to Standard Methods (1965) to obtain information, together with the chronic tests, for calculating application factors. Measured PCB concentrations were used for all calculations.

Full life-cycle studies with the fathead minnows, and the tests with Jordanella, were started with newly hatched young (< 24 hours old). The tests with Jordanella consisted of daily survival observations, with growth measurements at termination of the test after 30 days. Young fathead minnows were tested in the same diluter system, but different tanks, during the same time to get comparative information on the two species.

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

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

Gammarus tests. -- Juvenile scuds, Gammarus pseudolimnaeus, were obtained from the Eau Claire River (Wisconsin), and were held in the laboratory for at least a week prior to testing. Twenty animals were

placed in each tank, with two (1242 test) and three (1248 test) tanks for each PCB concentration, giving 40 (1242) and 60 (1248) scuds in each concentration.

They were fed a combination of well-soaked maple and aspen leaves, dry commercial fish food, and algae, which was permitted to grow on the test tanks prior to introduction of the scuds. The total length of the scud tests was two months; they were tested as juveniles and held under test conditons until they coupled and reproduced (one month). Reproduction was allowed to continue for one month before the test was terminated. The adults were counted and young counted and weighed.

Midge Tests. -- The test species was Tanytarsus dissimilis.

The midges occur as "guests" in most tests systems in the laboratory, and its original source is unknown.

The midges were reared in extra tanks in the test room so an abundance of egg carrying females were flying in the room at all times. These females deposited eggs in all clean test tanks when the test was started and within a few hours all tanks contained larve. As this was a continuing random ovipositing pattern an abundant supply of young midges was available. The occurrence of mature midge cases and pupal cases which the growing larvae constructed were utilized as a measure of growth and survival. The additional occurrence of pupae and cast pupal skins at the water surface indicated that adult emergence was or was not successful.

The test period was 3 weeks; at the end of this period all mature larval cases and pupal cases were counted. Test tanks were observed daily to determine if successful adult emergence was occurring. This procedure was useful under the particular conditions of these tests but is not recommended as a routine method.

ANALYTICAL RESULTS

Extraction efficiencies of this method are shown in Table 2. These recovery values based on the single peak evaluation, referred to above, show little difference in the Aroclors. This would indicate that the extraction of the compound represented by the peak may be only slightly affected by the other compounds present.

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

Tables 3, 4, and 5 show the comparison of nominal and measured concentrations. Comparing the percent nominal values for any series of dilutions gives some indication of how well that diluter system was working. It can be seen that except for a few high values the systems were working well.

Table 2. Percent Recovery of Aroclor Compounds During Extraction from Test Water

Aroclor	1242	1248	1254
Mean	89	86	82
Standard deviation	5	8	7
Range	81-96	71-98	71-96
Number of samples	12	23	18

Table 3. Comparison of Nominal and Measured Concentrations of Aroclor 1242.

Test	Nominal Conc.	Mean Measured Conc.	Standard Deviation	Range	Mean Percent of Nominal	Number of Samples	Total Samples
I	300	234	13	210-250	78	8	
	100	81	7.4	75-89	81	3	
	33	26*	--	-	--	-	
	11	8.7*	--	-	--	-	
	3.5	2.8*	--	-	--	-	
							11
II	75	51	7.6	39-58	68	6	
	25	15	2.3	13-19	61	6	
	8.3	5.4	1.3	3.9-7.3	65	14	
	2.8	2.9	.90	1.5-4.4	105	11	
	.93	.86	.32**	.52-1.2	93	2	
							39
I and II							50

* Calculated - based on mean percent (of nominal) recovery.

** This value is 1/2 the range.

Table 4. Comparison of Nominal and Measured Concentrations of Aroclor 1248.

Test	Nominal Conc.	Mean Measured Conc.	Standard Deviation	Range	Mean Percent of Nominal	Number of Samples	Total Samples
I	75	40	6.2	34-48	53	4	
	25	14	4.1	9.1-17	54	4	
	8.3	5.8	.25**	5.5-6.0	69	2	
	.93	.37	.02**	.35-.39	40	2	12
II	30	18	1.5	17-21	61	5	
	10	5.1	3.0	2.6-6.7	51	10	
	3.3	2.2	.30	1.7-2.6	65	9	
	1.1	.54	.096	.47-.65	49	3	
	.37	.18	.021	.16-.20	50	3	30
III	20.0	7.5*	--	--	--	-	
	6.7	2.5	.18	2.3-2.8	37	6	
	2.2	.86	.31	.51-1.3	39	5	
	.74	.26	.12	.10-.37	36	4	
	.25	.10	--	--	--	-	15
I, II, and III							57

* Calculated - based on mean percent (of nominal) recovery.

** These values are 1/2 the range.

Table 5. Comparison of Nominal and Measured Concentrations of Aroclor 1254.

Test	Nominal Conc.	Mean Measured Conc.	Standard Deviation	Range	Mean Percent of Nominal	Number of Samples	Total Samples
I	100	33	--	--	33	1	
	33	9.0	.35**	8.6-9.3	27	2	
	11	3.5	.60	2.9-4.1	32	2	
	3.7*	1.2*	--	--	--	-	
	1.2	.45	--	--	38	1	6
II	30	9.0	.44	8.7-9.5	30	3	
	10	3.8	.58	3.5-4.5	38	3	
	3.3	1.7	--	--	51	1	
	1.1	.92	--	--	84	1	
	.37*	--	--	--	--	-	8
III	25	15	2.5	12-17	58	2	
	8.3	4.6	.68	3.6-5.7	55	14	
	2.8	1.8	.29	1.4-2.2	62	15	
	.93	.52	.12	.38-.69	58	8	
	.31	.23	.02**	.21-.25	75	2	41
I, II, and III							55

* Calculated - based on mean percent (of nominal) recovery.

** These values are 1/2 the range.

BIOLOGICAL RESULTS

Daphnia Static Tests

Aroclor 1248 was most toxic to Daphnia with a 3-week LC50 of 25 µg/liter. Fifty percent reproductive impairment occurred at 24 µg/l, with 16 percent impairment at 16 µg/l. The toxicity of the other PCB's progressively decreased (Table 6) from 1248 to 1221 and from 1248 to 1268. The Aroclors 1254, 1260, and 1262 were nearly as toxic as 1248, with 1242 and 1232 being about 1/2 as toxic. Aroclor 1221 was much less toxic with an LC50 of 180 µg/l. The least toxic was 1268, with an LC50 of 253 µg/l (Table 6).

In all tests reproduction, as judged by the number of young produced, was the most sensitive indicator of toxicity. Values for 50 percent reproductive impairment of all PCB's tested were lower (Table 6) than the LC50 for survival. The confidence limits however, indicate that they are not significantly different except for 1221 and 1268.

No survival or reproduction occurred at 40 percent of the PCB mixture after three weeks. At 20 percent of the mixture there was 17 percent adult survival and 13 percent young survival (Table 7). Fifty percent of the adults survived at 15 percent of the mixture. Young survival was reduced to 50 percent at 14 percent of the mixture, with 16 percent reproductive impairment occurring at 11 percent of the mixture. Good survival, growth, and reproduction occurred at and below 5 percent of the mixture. The toxicity of the separate PCB's appears to be additive under the conditions of these tests.

Table 6. The Comparative Toxicity of Eight Aroclors[a] (in µg/liter) to Daphnia magna in Lake Superior Water as Determined in Static Test Conditions

Aroclor	Three Week LC50	Confidence Limits[b]	Reproductive Impairment 50%	Confidence Limits	16%	Confidence Limits
1221	180	158-205	125	116-135	89	85.6-92.6
1232	72	62.6-82.8	66	60-72.6	53	50.5-55.7
1242	67	55.4-81	63	56.3-70.5	48	45.2-50.9
1248	25	21.4-29.2	24	21.2-27.1	16	13.9-18.4
1254	31	25.8-37.2	28	23.1-33.9	18	14.5-22.3
1260	36	27.7-46.8	33	27.5-39.6	22	17.7-27.3
1262	43	37-49.9	41	33-53.3	24	17.6-32.6
1268	253	222-288	206	185-228	162	146-179

[a]Polychlorinated Biphenyls: Aroclor 1221-1268. Monsanto Chemical Company, St. Louis, Mo

[b]Ninety-five percent confidence limits.

Table 7. Survival and Reproduction of *Daphnia magna* After 3 Weeks' Exposure to Various Percentages of a PCB Mixture of 16% Impairment Concentrations[1].

Percent of Mixture[2]	Initial Number of Animals	Adults Alive at End of Test			Mean Percent Survival of Adults	Total Young Produced			Mean Young Produced as Percent of Control[3]
		Test 1	Test 2	Test 3		Test 1	Test 2	Test 3	
40%	20	0	0	0	0%	0	0	0	0%
20%	20	2	8	0	17%	80	152	68	13%
10%	20	16	20	20	93%	554	792	631	87%
5%	20	18	20	20	97%	690	795	804	100%
1%	20	14	19	19	87%	425	670	668	77%
0.5%	20	15	20	20	92%	566	871	701	94%
0.0% Control	20	17	18	20	92%	688	757	836	99.5%

[1] That concentration which permitted reproduction but reduced it by 16%, for each Aroclor, was added together. Various percentages of the mixture were then tested.

[2] Mixture consisted of the following concentrations (in µg/liter): 1221 = 87, 1232 = 53, 1242 = 48, 1248 = 16, 1254 = 18, 1260 = 22, 1262 = 24, and 1268 = 162.

[3] Percent decrease from the highest young produced in either the controls or one of the lower tested toxicant levels.

Daphnia Continuous-Flow Tests

Aroclor 1254 was most toxic to *Daphnia* under continuous-flow test conditions. There was no significant difference between the two-week LC50 of 1.8 µg/liter and the three-week LC50 of 1.3 µg/liter (Table 8). Reproductive impairment occurred at or just below those levels which prevented survival of the adults. Aroclor 1248 killed 50 percent of the adult *Daphnia* at 2.6 µg/liter; with 50 percent survival of the young at 2.1 µg/liter. Sixteen percent reproductive impairment ocurred at 1.0 µg/liter for 1248 and for 1254 at 0.48 (2 weeks) and 1.0 (3 weeks) µg/liter (Table 8). Almost no reproduction occurred at 7.5 µg/liter 1248 (Table 9). No reproduction occurred at 3.8 µg/liter 1254 (Table 10) with no adults surviving 3.5 µg/liter (Table 11). The number of young produced per initial adult and per surviving adult were determined (Table 12 and 13). This ranged from 0.7 young/initial adult in 7.5 µg/liter 1248 to 76 young/surviving adult in .45 µg/liter 1254 (Table 14).

Table 8. Calculated 2- and 3-week LC50 values, and 50 percent and 16 percent reproductive impairment[1] for Daphnia magna subjected to Aroclor 1248 and Aroclor 1254 in continuous-flow conditions.

PCB	2 or 3 week LC50 (µg/liter)	Reproductive Impairment (µg/liter) 50%	16%
1248	2.6 (2 weeks)	2.1	1.0
1254	1.8 (2 weeks)	1.1	.48
1254	1.3 (3 weeks)	1.3	1.0

[1] Reproductive impairment is defined as percent decrease from the highest young produced in either the controls or one of the lower tested toxicant levels

Table 9. Survival and reproduction of *Daphnia magna* after 2 weeks' continuous-flow exposure to Aroclor 1248.

Concentration (µg/l)	Test Tanks	Initial Number of Animals	Adults Alive at End of Test			Total Young Produced		
			Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
7.5	A	5	0	1	0	0	28	0
	B	5	0	0	1	0	0	0
	C	5	0	0	0	0	0	0
	D	5	0	1	0	0	15	0
2.5	A	5	0	4	2	0	159	12
	B	5	4	4	2	224	12	3
	C	5	5	5	3	209	0	22
	D	5	0	5	5	0	30	28
.86	A	5	5	5	4	150	298	145
	B	5	4	5	5	143	86	12
	C	5	5	4	5	258	48	64
	D	5	5	5	3	57	38	42
.26	A	5	4	3	4	231	118	110
	B	5	4	5	5	229	0	97
	C	5	5	5	4	279	0	53
	D	5	5	5	3	278	214	32
.1	A	5	5	3	3	0	0	38
	B	5	4	4	3	57	0	52
	C	5	-	5	4	--	89	120
	D	5	4	2	-	193	78	--
0.0 Control	A	5	4	2	3	61	0	66
	B	5	-	3	2	--	44	16
	C	5	4	4	2	93	183	28
	D	5	4	2	-	238	57	--

Table 10. Survival and reproduction of Daphnia magna after 2 weeks' continuous-flow exposure to Aroclor 1254.

Mean Measured Concentration (ug/l)	Test Tanks	Initial Number of Animals	Adults Alive at End of Test		Total Young Produced	
			Test 1	Test 2	Test 1	Test 2
9.0	A	5	0	0	0	0
	B	5	0	0	0	0
3.8	A	5	0	0	0	0
	B	5	0	0	0	0
1.7	A	5	4	4	0	109
	B	5	0	4	0	130
.92	A	5	4	5	49	127
	B	5	5	-	93	---
.37	A	5	4	4	36	239
	B	5	-	4	--	229
0.0 Control	A	5	4	2	52	122
	B	5	-	2	--	116

Table 11. Survival and reproduction of Daphnia magna after 3 weeks' continuous-flow exposure to Aroclor 1254.

Mean Measured Concentration (µg/l)	Test Tanks	Initial Number of Animals	Adults Alive at End of Test		Total Young Produced	
			Test 1	Test 2	Test 1	Test 2
33	A	5	0	0	0	0
	B	5	0	0	0	0
	C	5	0	0	0	0
	D	5	0	0	0	0
9.0	A	5	0	0	0	0
	B	5	0	0	0	0
	C	5	0	0	0	0
	D	5	0	0	0	0
3.5	A	5	0	0	0	0
	B	5	0	0	0	0
	C	5	0	0	0	0
	D	5	0	0	0	0
1.2	A	5	4	4	254	152
	B	5	3	4	216	155
	C	5	4	3	452	105
	D	5	2	4	131	118
.45	A	5	3	5	311	351
	B	5	0	5	172	372
	C	5	3	5	328	159
	D	5	-	3	---	123
0.0 Control	A	5	4	5	172	246
	B	5	2	4	102	309
	C	5	5	4	136	189
	D	-	-	-	---	---

Table 12. Summary of Survival and Reproduction of Daphnia magna After 2 weeks' Continuous-Flow Exposure to Aroclor 1248.

Mean Measured Concentration (µg/l)	Initial Number of Animals	Total Number of Adults Alive at End of Tests	Mean Percent Survival of Adults	Total Young Produced	Young Produced as Percent[1] of Control	Young Per Initial Adult	Young Per Surviving Adult
7.5	60	3	5%	43	2.6%	0.7	14
2.5	60	39	65%	699	43%	12	18
.86	60	55	92%	1441	88%	24	26
.26	60	52	87%	1641	100%	27	31
.1	50	37	74%	627	46%	13	17
0.0 Control	50	30	60%	786	57%	16	26

[1] Percent decrease from the highest young produced in either the controls or one of the lower tested toxicant levels.

Table 13. Summary of Survival and Reproduction of Daphnia magna After 2 weeks' Continuous-Flow Exposure to Aroclor 1254.

Mean Measured Concentration (µg/l)	Initial Number of Animals	Total Number of Adults Alive at End of Tests	Mean Percent Survival of Adults	Total Young Produced	Young Produced as Percent[1] of Control	Young Per Initial Adult	Young Per Surviving Adult
9.0	20	0	0%	0	0%	0	0
3.8	20	0	0%	0	0%	0	0
1.7	20	12	60%	239	36%	12	20
.92	15	14	93%	269	53%	18	19
.37	15	12	80%	504	100%	34	42
0.0 Control	15	8	53%	290	58%	19	36

[1] Percent decrease from the highest young produced in either the controls or one of the lower tested toxicant levels.

Table 14. Summary of Survival and Reproduction of Daphnia magna After 3 weeks' Continuous-Flow Exposure to Aroclor 1254.

Mean Measured Concentration (µg/l)	Initial Number of Animals	Total Number of Adults Alive at End of Tests	Mean Percent Survival of Adults	Total Young Produced	Young Produced as Percent[1] of Control	Young Per Initial Adult	Young Per Surviving Adult
33	40	0	0%	0	0%	0	0
9.0	40	0	0%	0	0%	0	0
3.5	40	0	0%	0	0%	0	0
1.2	40	28	70%	1583	76%	39	56
.45	35	24	69%	1816	100%	52	76
0.0 Control	30	24	80%	1154	74%	38	48

[1] Percent decrease from the highest young produced in either the controls or one of the lower tested toxicant levels.

Fathead Minnow

Calculated 96-hour LC50 values were 7.7 µg/l for newly hatched young in 1254 and 15 µg/l for newly hatched young in 1242. The 96-hour LC50 for 2 month-old fatheads was greater than 234 µg/l 1242 (ca. 300 µg/l) and greater than 33 µg/l 1254.

Using the calculated 96-hour LC50 values for young fish and the safe levels determined from the long-term tests application factors of 0.2 for 1254 and 0.16 for 1242 were calculated. If LC50 values for juvenile fish were used the application factors would be much more restrictive.

Reproduction occurred at and below 1.8 µg/l Aroclor 1254 and at and below 5.4 µg/l Aroclor 1242. No survival or reproduction occurred at 4.6 µg/l 1254 or at 15 µg/l 1242, indicating that 1254 is the more toxic of the two PCB's. This is also shown by the lower 96-hour LC50 value for 1254.

Aroclor 1242 -- All fish were dead after 96 hours in 51 µg/l 1242; in the duplicate 85 percent were dead. Ninety-five percent in one duplicate were dead after eight days at 15 µg/l. Only 5 percent were dead after 60 days at 5.4 µg/l (Table 15). There was no significant difference in the final weights or total lengths of the fish (Tables 16 and 17) at the end of the test. Spawning results and egg production were highly variable (Table 18), even between duplicate tanks. Good spawning occurred in 5.4 µg/l in Tank A but no spawning occurred at all in 5.4 B, even though there were the same numbers of males and

females. A slightly higher rate of flow into tank B could account for the lack of spawning in Tank B because the cumulative nature of PCB's would cause an increase in tissue level of PCB's.

Egg hatchability and fry survival were also quite variable, although not so much between duplicates (Table 19). Good hatching occurred in 5.4 µg/l. Eggs produced by the controls but maintained at the higher concentrations of 15 and 51 µg/l hatched with good success but none of the fry survived at the high concentrations (Table 20), indicating that the egg stage is not affected by PCB concentrations which are rapidly lethal to newly hatched fry.

The percent survival of fry produced and reared at the same concentrations was excellent (Table 21) indicating that the long-term accumulation of PCB's is the biggest problem, at least at low levels that are not rapidly lethal.

Aroclor 1254 -- All fish were dead after 96 hours in 15 µg/l 1254 in both duplicate tanks. Twenty-five percent were dead after 17 days at 4.6 µg/l, and at 60 days fifty percent were dead at 4.6 µg/l. Survival at lower concentrations was not significantly different from the controls (Table 22). There was no significant difference in the mean terminal weights of the experimental fish (Table 23), however, there was a delay in growth (length) at 4.6 µg/l (Table 24). Spawning occurred at 1.8 µg/l but was significantly less than that in lower concentrations. Spawning was highly variable during the test, even between duplicate tanks (Table 25).

Egg hatchability and fry survival were good at and below 1.8 µg/l, with similar results in all tanks (Table 26). Eggs produced by the controls and maintained in higher PCB concentrations (15 µg/l) hatched readily

but all young were dead within 96 hours (Table 27). Young held for 30 days in separate tanks at the same PCB concentrations they were spawned at all survived and grew well (Table 28).

Aroclor 1248 -- The fathead minnows died rapidly at 18 µg/l and none were alive at the end of 30 days. Seventy-five percent were alive at 5.1 µg/l but their weight was only one-third that of the controls; though the final lengths were not significantly different (Table 29).

The final weight of the fish in 2.2 µg/l was only half that of the controls; final lengths were the same. The results in the lowest 2 concentrations were not significantly different from the controls.

Table 15. Survival of Fathead Minnows at Various Time Intervals (Aroclor 1242).

Mean Measured Concentration (µg/l)	Test Tanks	Initial Number of Animals	Survival at Various Time Intervals							
			4 Days Jan. 16--96 hrs.		8 Days Jan. 20		23 Days Feb. 4		60 Days March 12	
			No.	%	No.	%	No.	%	No.	%
51	A	20	3	15%	0	0%	0	0%	0	0%
	B	20	0	0%	0	0%	0	0%	0	0%
15	A	20	20	100%	1	5%	0	0%	0	0%
	B	20	20	100%	0	0%	0	0%	0	0%
5.4	A	20	20	100%	20	100%	19	95%	19	95%
	B	20	20	100%	20	100%	19	95%	19	95%
2.9	A	20	20	100%	20	100%	18	90%	15	75%
	B	20	20	100%	20	100%	18	90%	18	90%
.86	A	20	20	100%	20	100%	17	85%	17	85%
	B	20	20	100%	20	100%	19	95%	19	95%
0.0 Control	A	20	20	100%	20	100%	19	95%	16	80%
	B	20	20	100%	20	100%	16	80%	15	75%

Table 16. Mean Terminal Weight of Fathead Minnows (Aroclor 1242).

Mean Measured Concentration (µg/l)	Number of Males	Mean Weight (g)	Number of Females	Mean Weight (g)	Mean wt. Males and Females
51	0	0	0	0	0
15	0	0	0	0	0
5.4	8	2.40	11	1.05	1.64
2.9	9	2.17	9	.97	1.56
.86	6	2.30	4	.82	1.71
0.0 Control	4	1.70	14	1.10	1.31

Table 17. Growth (Length) of Fathead Minnows (Aroclor 1242).

Mean Measured Concentration (µg/l)	Mean Length of Fish (mm)				
	After 2 Months	After 3 Months	After 8 Months (End of Test)		
			Males	Females	Both
51	0	0	0	0	0
15	0	0	0	0	0
5.4	19.1	28.1	61.7	49.4	54.5
2.9	21.4	28.9	58.3	46.3	52.3
.86	21.1	28.5	58.5	45.5	53.3
0.0	20.6	28.4	57.9	47.9	50.1

Table 18. Spawning Results and Egg Production of the Fathead Minnow (Aroclor 1242).

Mean Measured Concentration (µg/l)	Test Tanks	Number of Males	Number of Females	Number of Spawnings	Number of Spawnings Per Female	Total Eggs Produced	No. of Eggs Per Spawning	No. of Eggs Per Female
51	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
15	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
5.4	A	4	5	25	5.0	1514	61	303
	B	4	6	0	0	0	0	0
2.9	A	5	4	24	6.0	1923	80	481
	B	4	5	9	1.8	424	47	85
.86	A	6	4	5	1.3	138	28	35
	B*	--	--	--	--	--	--	--*
0.0 Control	A	1	8	42	5.2	5350	127	669
	B	3	6	24	4.0	1288	54	215

* Accidentally killed

Table 19. Egg Hatchability and Fry Survival of the Fathead Minnow (Aroclor 1242).

Mean Measured Concentration (µg/l)	Test Tanks	Number of Eggs Used	Number of Live Fry Obtained	Egg Hatchability Percent
51	A	0	0	0%
	B	0	0	0%
15	A	0	0	0%
	B	0	0	0%
5.4	A	150	121	81%
	B	0	--	--
2.9	A	125	37	30%
	B	160	76	47%
.86	A	108	91	84%
	B*	--	--	--
0.0 Control	A	350	216	62%
	B	75	33	44%

* Accidentally killed

Table 20. Egg Hatchability and Fry Survival of Eggs Produced by Control Fathead Minnows but Maintained in Higher PCB Concentrations (Aroclor 1242).

Mean Measured Concentration (ug/l)			Number of Eggs Used	Egg Hatchability Percent	Number of Live Fry Obtained	Fry Survival After 30 Days Percent
Source of Eggs	Test Tanks	Eggs Exposed to				
Control	A	15 µg/l	25	60%	15	0%
Control	A	51 µg/l	50	82%	41	0%
Control	B	51 µg/l	35	91%	32	0%

Table 21. Results of 30-day Growth and Survival Study of Young Fathead Minnows (Aroclor 1242).

Mean Measured Concentration (ug/l)	Test Tanks	Number of Fry Used	Number of Fry Surviving	Percent Survival	Mean Dry Weight (mg)	Mean Length (mm)
51	A*	0	0	0%	0	0
	B*	0	0	0%	0	0
15	A*	0	0	0%	0	0
	B*	0	0	0%	0	0
5.4	A	110	93	84%	12.8	19.2
	B*	0	--	--	--	--
2.9	A	15	14	93%	25.3	21.2
	B	46	42	91%	16.3	21.4
0.0 Control	A	30	23	77%	8.0	17.2
	B	16	15	94%	46.9	27.7

* No fry produced at these concentrations

Table 22. Survival of Fathead Minnows at Various Time Intervals (Aroclor 1254).

Mean Measured Concentration (µg/l)	Test Tanks	Initial Number of Animals	Survival at Various Time Intervals					
			4 Days Jan. 29--96 hrs.		17 Days Feb. 11		60 Days March 26	
			No.	%	No.	%	No.	%
15	A	20	0	0%	0	0%	0	0%
	B	20	0	0%	0	0%	0	0%
4.6	A	20	20	100%	16	80%	10	50%
	B	20	20	100%	14	70%	11	55%
1.8	A	20	20	100%	18	90%	17	85%
	B	20	20	100%	18	90%	16	80%
.52	A	20	20	100%	17	85%	18	90%
	B	20	20	100%	13	65%	13	65%
.23	A	20	20	100%	18	90%	17	85%
	B	20	20	100%	19	95%	16	80%
0.0 Control	A	20	20	100%	18	90%	19	95%
	B	20	20	100%	16	80%	16	80%

Table 23. Mean Terminal Weight of Fathead Minnows (Aroclor 1254).

Mean Measured Concentration (µg/l)	Number of Males	Mean Weight (g)	Number of Females	Mean Weight (g)	Mean wt. Males and Females
15	0	0	0	0	0
4.6	5	2.42	10	1.35	1.7
1.8	7	2.09	14	1.28	1.55
.52	8	2.67	12	1.25	1.82
.23	6	2.66	13	1.38	1.65
0.0 Control	7	2.8	12	1.51	1.93

Table 24. Growth (Length) of Fathead Minnows (Aroclor 1254).

Mean Measured Concentration (µg/l)	Mean Length of Fish (mm)				
	After 2 Months	After 3 Months	After 8 Months (End of Test)		
			Males	Females	Both
15	0	0	0	0	0
4.6	16.4	30.2	60.0	50.6	53.7
1.8	23.5	32.9	59.4	50.9	53.8
.52	27.2	35.5	63.8	51.8	56.6
.23	26.4	35.1	61.2	51.2	54.4
0.0	24.9	33.6	62.8	51.9	55.9

Table 25. Spawning Results and Egg Production of the Fathead Minnow (Aroclor 1254).

Mean Measured Concentration (ug/l)	Test Tanks	Number of Males	Number of Females	Number of Spawnings	Number of Spawnings Per Female	Total Eggs Produced	No. of Eggs Per Spawning	No. of Eggs Per Female
15	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
4.6	A	4	5	0	0	0	0	0
	B	1	5	0	0	0	0	0
1.8	A	3	7	14	2.0	1473	105	210
	B	4	7	1	0.14	22	22	3
.52	A	4	6	30	5.0	4142	138	690
	B	4	6	33	5.5	2538	77	423
.23	A	1	8	35	4.4	2993	86	374
	B	5	5	8	1.6	346	43	69
0.0 Control	A	3	6	27	4.5	2838	105	473
	B	4	6	2	0.3	206	103	34

Table 26. Egg Hatchability and Fry Survival of the Fathead Minnow (Aroclor 1254).

Mean Measured Concentration (µg/l)	Test Tanks	Number of Eggs Used	Number of Live Fry Obtained	Egg Hatchability Percent
15	A	0	0	0%
	B	0	0	0%
4.6	A	0	0	0%
	B	0	0	0%
1.8	A	300	186	62%
	B	50	48	96%
.52	A	375	251	67%
	B	345	205	59%
.23	A	272	150	55%
	B	0	0	0%
0.0 Control	A	250	191	76%
	B	150	107	71%

Table 27. Egg Hatchability and Fry Survival of Eggs Produced by Control Fathead Minnows but Maintained in Higher PCB Concentrations (Aroclor 1254).

Source of Eggs	Mean Measured Concentration (µg/l) Test Tanks	Eggs Exposed to	Number of Eggs Used	Egg Hatchability Percent	Number of Live Fry Obtained	Fry Survival After 96 Hrs. Percent
Control	A	15 µg/l	25	84%	21	0%
Control	B	15 µg/l	25	88%	22	0%
Control	B	15 µg/l	50	94%	47	0%
Control	A	15 µg/l	50	100%	50	0%

Table 28. Results of 30-day Growth and Survival Study of Young Fathead Minnows (Aroclor 1254).

Mean Measured Concentration (ug/l)	Test Tanks	Number of Fry Used	Number of Fry Surviving	Percent Survival	Mean Dry Weight (mg)	Mean Length (mm)
15	A	0	0	0%	0	0
	B	0	0	0%	0	0
4.6	A	0	0	0%	0	0
	B	0	0	0%	0	0
1.8	A	60	55	92%	18.05	19.85
	B	0	--	--	--	--
.52	A	30	21	70%	16.1	19.9
	B	10	10	100%	30.8	24.2
.23	A	74	54	73%	18.0	19.15
	B	0	--	--	--	--
0.0 Control	A	56	55	98%	21.3	19.8
	B	24	24	100%	16.3	20.9

Table 29. Results of 30-day Survival and Growth Study of Newly Hatched Fathead Minnows (Aroclor 1248).

Mean Measured Concentration (µg/l)	Initial Number of Animals	Mean Percent Survival	Final Mean Weight (g)	Final Mean Length (mm)
18	20	0%	-	-
5.1	20	75%	.36	17.9
2.2	20	85%	.49	19.1
.54	20	80%	.92	20.8
.18	20	100%	1.47	20.3
0	20	85%	1.11	18.4

Jordanella

Aroclor 1248 -- The fish held in 18 µg/l lived for about two weeks before they started to die. One died every 2-3 days until the test was terminated (40 days); at which time all were dead. Thirty-five percent were alive at 5.1 µg/l at the end of the test, with mean weight only 15 percent that of the controls (Table 30). The mean length was 21.8 mm, compared to 24.6 for the controls.

The fish in 18 and 5.1 µg/l exhibited almost total loss of fins and tail, as though they had been eroded away. The one remaining fish at 18 µg/l and 3 fish in 5.1 µg/l died due to a temperature shock when the temperature dropped from 25° C to 21° C, indicating the precarious position they were in even though they were still alive.

Table 30. Results of 40-day Survival and Growth Study of Newly Hatched Jordanella floridae (Aroclor 1248).

Mean Measured Concentration (µg/l)	Initial Number of Animals	Mean Percent Survival	Final Mean Weight (g)	Final Mean Length (mm)
18	20	0%	-	-
5.1	20	35%	.60	21.8
2.2	20	85%	3.02	24.1
.54	20	100%	4.47	26.3
.18	20	90%	3.90	25.5
0	20	100%	4.33	24.6

Gammarus

Ninety-six hour LC50 values were calculated for Aroclor 1242 and 1248. Aroclor 1248 was twice as toxic as 1242 with an LC50 of 29 µg/l for 1248 and 73 µg/liter for 1242 (Table 31).

Survival of the initial test animals after 30 days was significantly less than survival after 96 hours (Tables 32 and 33). No live animals remained at 26 µg/l 1242 or 18 µg/l 1248. Fifty-three percent were alive at 8.7 µg/l 1242 (Table 34) and 52 percent were alive at 5.1 µg/l 1248 (Table 35).

Good reproduction occurred at 2.8 µg/l 1242 and 2.2 µg/l 1248. Some reproduction occurred at 5.1 µg/l 1248 but it was only half that of the control and only one-fourth that of 2.2 µg/l.

Application factors (Mount and Stephan, 1967) of 0.1 for 1242 and 0.15 for 1248 were calculated for the PCB's and Gammarus.

Table 31. Calculated 96-hour LC50 Values for Gammarus pseudolimnaeus Subjected to Aroclor 1242 and Aroclor 1248[1]

Aroclor (PCB)	Test	Individual Test LC50 Values (µg/liter)	Mean LC50 (µg/liter)
1242	1	72	73
	2	74	
1248	1	26	
	2	28	29
	3	30	
	4	32	

[1] Calculated according to Standard Methods, 1971 (APHA).

Table 32. Survival and Reproduction of Gammarus pseudolimnaeus After 2 Months' Exposure to Aroclor 1242.

Mean Measured Concentration (µg/l)	Test Tanks	Initial Number of Animals	Adults Alive at End of Test	Total Young Produced
234	A	20	0	0
	B	20	0	0
81	A	20	0	0
	B	20	0	0
26	A	20	0	0
	B	20	0	0
8.7	A	20	11	0
	B	20	10	0
2.8	A	20	14	36
	B	20	17	95
0.0 Control	A	20	7	53
	B	20	12	77

Table 33. Survival and Reproduction of Gammarus pseudolimnaeus After 2 Months' Exposure to Aroclor 1248.

Mean Measured Concentration (µg/l)	Test Tanks	Initial Number of Animals	Adults Alive at End of Test	Total Young Produced	Total Weight of Young (mg)
18	A	15	0	0	0
	B	15	0	0	0
	C	15	0	0	0
5.1	A	15	10	24	16
	B	15	10	94	28
	C	15	4	51	46
2.2	A	15	12	85	46
	B	15	10	284	140
	C	15	11	260	101
.54	A	15	6	130	103
	B	15	13	211	132
	C	15	13	286	101
.18	A	15	7	83	39
	B	15	12	230	105
	C	15	14	44	10
0.0 Control	A	15	10	169	91
	B	15	10	42	15
	C	15	9	119	94

Table 34. Summary of Survival and Reproduction of Gammarus pseudolimnaeus After 2 Months' Exposure to Aroclor 1242.

Mean Measured Concentration (µg/l)	Initial Number of Animals	Total Number of Adults Alive at End of Test	Mean Percent Survival of Adults	Total Young Produced	Young Per Surviving Adult
234	40	0	0	0	0
81	40	0	0	0	0
26	40	0	0	0	0
8.7	40	21	52%	0	0
2.8	40	31	77%	131	4.2
0.0 Control	40	19	48%	130	6.8

Table 35. Summary of Survival and Reproduction of Gammarus pseudolimnaeus After 2 Months' Exposure to Aroclor 1248.

Mean Measured Concentration (ug/l)	Initial Number of Animals	Total Number of Adults Alive at End of Test	Mean Percent Survival of Adults	Total Young Produced	Young Per Surviving Adult	Total Weight of Young (mg)	Mean Weight of Each Young Scud (mg)
18	45	0	0	0	0	0	0
5.1	45	24	53%	169	7.0	90	.53
2.2	45	33	73%	729	22.1	287	.39
.54	45	32	71%	627	19.6	336	.54
.18	45	33	73%	357	10.8	154	.43
0.0 Control	45	29	64%	330	11.4	200	.61

Table 35a. 1248 Gammarus Chronic - Statistical Treatment of Table 35.

Mean Measured Concentration (ug/l)	Mean % Survival	Total Young	Young/ Adult	Total Wt. Young
18	0	0	0	0
5.1	53.6±* 23.1	56± 35	8.2± 5.3	30± 15
2.2	73.3±** 6.5	210±* 108	19.7±* 11.2	96± 47
.54	71.3±** 27.1	209±* 78	20.0±* 3.3	112±* 17
.18	73.3±** 23.7	119± 98	11.4± 8.1	51± 49
0.0 Control	64.7±** 4.0	110± 64	11.4± 6.5	67± 45
Tukey's .05	49.4	206	18.7	95.9
.01	64.0	267	24.2	124

* Significantly different from 18 µg/l conc. at the 0.05 level.

** Significantly different from 18 µg/l conc. at the 0.01 level.

Note: In only one case (mean % survival) was the 18 µg/l treatment significantly different from the controls.

Midges

Adults emerged at concentrations up to 9 µg/liter 1248. Larvae were present at 18 µg/l but adult emergence did not occur. Abundant emergence did not occur above 5.1 µg/liter. Aroclor 1254 was more toxic to midges as no emergence occurred above 3.5 µg/l, and abundant emergence did not occur above 3 µg/liter, even though larvae were present (Table 36).

The survival and growth of the midge when tested with Aroclor 1254 was excellent in control chambers but was reduced by 50 percent at the lowest test concentration of .45 µg/liter (Table 36). At 1.2 µg/liter larval cases were reduced to 35 percent of the control and pupal cases were reduced to 24 percent of the control. No larval cases were formed at 33 µg/l and no pupal cases were constructed at 9 µg/l. The calculated 2-week LC50 for 1254(50 percent reduction based on control as 100 percent was .65 µg/l for larvae and .45 µg/l for pupae, indicating that a safe level for midge well-being is below 1 µg/liter of Aroclor 1254.

Table 36. Effect of Aroclor 1254 on the Growth and Survival of the Midge Tanytarsus dissimilis.

Mean Measured Concentration (µg/l)	Test	No. of Mature Larval Cases			No. of Pupal Cases		
		Each Test	Mean	% of Control	Each Test	Mean	% of Control
33	A	0			0		
	B	0	0	0%	0	0	0%
	C	0			0		
	D	0			0		
9.0	A	0			0		
	B	0	.25	.2%	0	0	0%
	C	0			0		
	D	1			0		
3.5	A	3			1		
	B	7	24	22%	0	3	10%
	C	14			5		
	D	-			-		
1.2	A	43			7		
	B	68	37	35%	5	7	24%
	C	4			2		
	D	35			7		
.45	A	70			4		
	B	25	56	52%	27	16	55%
	C	82			16		
	D	48			16		
0.0 Control	A	94			32		
	B	108	107	100%	33	29	100%
	C	119			22		
	D	-			-		

DISCUSSION

Analytical Results

The method described here worked very well for monitoring the flowing system being used. The variability of this method, as indicated by the variability in analysis of duplicate samples mentioned above, is less than that of the test organisms. It was reasoned that the variability of the test organisms or diluter system would be greater than that of the total analysis so some accuracy was sacrificed in the interest of time. The fact that our test water (Lake Superior untreated) is relatively free of organics, in amounts that would interfere with a G. C. analysis, allowed us to eliminate a cleanup step thereby saving time and reducing the manipulative error. However, a cleanup step is recommended when extracting volumes of water larger than those used here or when using the more sensitive Electron Capture Detectors.

A residue chemist using this method for anything other than monitoring a closed system, such as ours, would find that the sacrifices in accuracy made to reduce the time of analysis could not be tolerated. This method could not, then, be used in monitoring natural bodies of water or tissue from affected organisms.

Daphnia

Daphnia magna was used as a test organism because its small size, short life cycle, and sensitivity to chlorinated hydrocarbons makes it ideally suited to determine the comparative toxicity of related chemicals such as the Aroclor mixtures. The fact that it is a significant representative of a large group of zooplankton important as fish food makes the information more useful in establishing cirteria for safe-guarding aquatic life from PCB's.

Because the continuous-flow tests indicated much lower safe levels for Daphnia we believe that the static test method should not be used for establishing water quality criteria for PCB's, even though it is a useful technique for determining relative toxicity. Continuous-flow systems should be employed whenever possible.

As can be seen in all of the tables there is a consistent trend for the mid-concentrations to have better survival and young production than the lowest test concentrations or the controls. This also has been observed by Biesinger and Christensen (1972) with Daphnia and summarized and discussed by Smyth (1967) with other animals tested with similar methods.

Fish

The results obtained from this study indicate that Polychlorinated Biphenyls are acutely toxic, but exhibit a much greater chronic toxicity at very low levels caused by the cumulative nature of the mixtures. The newly hatched larvae appear to be the most sensitive stage in the life cycle, at least for short-term exposure. Tissues of the fish used during this study are being analyzed by Dr. D. Stalling, Columbia, Mo., and should shed additional light on the relationship between toxicity of water concentrations and tissue levels. Because only the least sensitive of the fish tested survived to reproduce it appears that the newly hatched larvae are most sensitive to PCB's. Further generation studies with the resistant offspring might well produce young surviving higher concentrations than their parents were capable of spawning in. The eggs were apparently quite resistant or impermeable to the PCB mixtures. The PCB mixtures appeared to inhibit fungus growths on the incubating eggs, giving much better egg hatch at higher PCB levels. Juvenile fish, when tested for up to 30 days were also much less susceptible than very young fish.

Gammarus

The 96-hour LC50 values with Gammarus pseudolimnaeus indicate that PCB's are toxic after short periods of time, but the cumulative nature of these chlorinated hydrocarbon chemicals makes it more difficult to predict their toxicity from these short tests. The application factors of 0.16 and 0.20 may possibly be used to estimate long term safe levels from short tests with the other PCB's not tested with Gammarus.

Results from this study are in general agreement with the few studies that have been conducted with aquatic crustacea. Saunders (in press) found that 1242 killed G. fasciatus at 42 µg/l in 4- to 7-day tests, essentially the same as the value of 29 µg/liter determined during this study. However, Saunders obtained a 96-hour LC50 for 1254 of 2,400 µg/l which is clearly different than the 73 µg/l LC50 obtained during this study. Static tests conducted by Wildish (1970) with the scud Gammarus oceanicus and Aroclor 1254 indicated a range in toxicity from 100 to 1.0 µg/liter, similar to the values in this study with G. pseudolimnaeus.

The glass shrimp Palaemonetes kadiakensis was sensitive to 1254 under continuous-flow conditions with a 7-day LC50 of 3 µg/l (Saunders, in press). The crayfish Orconectes nais was more tolerant (Saunders, in press), with a 7-day LC50 of 100 µg/l 1254 in static tests and 80 µg/l 1254 under continuous-flow conditions. It had a 7-day LC50 of 30 µg/l when tested with 1254.

The pink shrimp tested by Duke (personal communication) showed the same delayed mortality as the Gammarus in the present study. The toxicity of Aroclor 1254 ranged from .94 µg/l for juveniles (51 percent after 15 days) to 3.5 µg/l after 35 days with adults. Regardless of the Aroclor concentration in the water, shrimp showed delayed mortality and died at a rate of one to two per day with no apparent symptoms of poisoning, just as Gammarus responded in the present study.

Midges

No reproductive data with insects is available to compare with the midge data in this study. However, Schoettger (personal communication) reports some acute data for other aquatic insects. Dragonfly and damselfly nymphs, Macromia sp. and Ischnura verticalis, were comparatively tolerant to 1242 and 1254. The dragonfly had a 7-day TL50 (static test) of 800 µg/l for 1242 and 1,000 µg/l for 1254. The damselfly, tested under continuous-flow conditions had a 4-day TL50 of 400 µg/l for 1242 and 200 µg/l for 1254.

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LITERATURE CITED

American Public Health Association. 1965. Standard Methods for the examination of Water and Wastewater. 12th ed. 769 pp.

Armour, J. A., and J. A. Burke. 1969. Polychlorinated Biphenyls as Potential Interference in Pesticide Residue Analysis. F. D. A. Laboratory Information Bull. No. 918, 11 p.

Armour, J. A., and J. A. Burke. 1970. Method for Separating Polychlorinated Biphenyls from DDT and its Analogs. JAOAC 53, 761.

Biesinger, K. E., and G. M. Christensen. 1972. Toxicity of Heavy Metals to Daphnia magna (in press).

Brungs, W. A. 1969. Chronic Toxicity of Zinc to the Fathead Minnow Pimephales promelas Rafinesque. Trans. Amer. Fish. Soc. 98(2): 272-279.

Cutkomp, L. K., and R. B. Koch. 1971. The Sensitivity of Fish ATPases to Polychlorinated Biphenyls. Nature (in press). (Dept. of Entomology, University of Minnesota 55101).

Duke, T. W. (personal communication). Gulf Breeze Lab., EPA, Gulf Breeze, Florida.

Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr., 1970. A Polychlorinated Biphenyl (Aroclor 1254) in the Water, Sediment, and Biota of Escambia Bay, Florida. Bull. Env. Cont. and Tox. 5(2):171-180.

Jensen, S. 1966. Report of a New Chemical Hazard. New Sci. 32, 612.

Jensen, S., R. G. Johnels, S. Olsson, and G. Otterlind. 1969. DDT and PCB in Marine Animals from Swedish Waters. Nature 224, 247.

Koeman, J. H., M. C. Ten Noever de Brauw, and R. H. de Vos. 1969. Chlorinated Biphenyl in Fish, Mussels, and Birds from the River Rhine and the Netherlands Coastal Area. Nature, 221:1126-1128.

Litchfield, J. T., and F. Wilcoxon. 1949. A simplified Method of Evaluating Dose-effect Experiments. J. Pharmacol. Exp. Ther. 96(2):99-113.

McKim, J. M., and D. A. Benoit. 1971. Effects of Long-Term Exposures to Copper on Survival, Growth, and Reproduction of Brook Trout (Salvelinus fontinalis). J. Fish. Res. Bd. Canada 28(5):655-662.

Mount, D. I., and W. A. Brungs. 1967. A Simplified Dosing Apparatus for Fish Toxicology Studies. Wat. Res. 1:21-29.

Mount, D. I., and C. E. Stephan. 1967. A Method for Establishing acceptable toxicant limits for Fish-Malathion and the Butoxyethanol Ester of 2,4-D. Trans. Amer. Fish. Soc. 96(2):185-193.

Peakall, D. B., and J. L. Lincer. 1970. Polychlorinated Biphenyls. Another Long-Life Widespread Chemical in the Environment. Bioscience 20(17):958-964.

Pesticide Analytical Manual, Vol. I. 1968. Food and Drug Administration, Washington, D.C.

Reynolds, L. M. 1969. Polychlorobiphenyls (PCB's) and Their Interference with Pesticide Residue Analysis. Bull. Environ. Contam. and Toxicol. 4, 128.

Risebrough, R., and V. Brodine. 1970. More Letters in the Wind. Environment 12(1):16-27.

Risebrough, R. W., P. Reiche, D. B. Peakall, S. G. Herman, and M. N. Kirven. 1968. Polychlorinated Biphenyls in the Global Ecosystem. Nature 220, 1098.

Risebrough, R. W., P. Reiche, and H. S. Olcott. 1969. Current Progress in the Determination of Polychlorinated Biphenyls. Bull. Env. Cont. Tox. 4, 192.

Saunders, H. O. (in press). Special Report on PCB's, In: Progress in Sport Fishery Research, Bureau of Sport Fisheries and Wildlife, Resource Publication USGPO.

Schoettger, R. A. (personal communication). Fish-Pesticide Lab., Columbia, MO.

Smyth, H. F., Jr. 1967. Sufficient Challenge. Fd. Cosmet. Toxicol. 5:51-58.

Sprague, J. B. 1969. Measurement of Pollutant Toxicity to Fish. I. Bioassay Methods for Acute Toxicty. Wat. Res. 3:793-821.

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

Stalling, D. L. (in press). Polychlorinated Biphenyls (PCB's) in Freshwater Ecosystems. Fish Pesticide Lab. Columbia, MO.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York. 481 p.

Veith, G. D., and G. F. Lee. 1970. A Review of Chlorinated Biphenyl Contamination in Natural Waters. Wat. Res. 4:265-269.

Wildish, D. J. 1970. The Toxicity of Polychlorinated Biphenyls (PCB) in Sea Water to Gammarus oceanicus. Bull. Env. Contam. and Toxicology, 5(3):202-204.

Zitco, V. 1970. Polychlorinated Biphenyls (PCB) Solubilized in Water by Nonionic Surfactants for Studies of Toxicity to Aquatic Animals. Bull. Env. Cont. Tox. 5(3):279-285.