Separate and simultaneous bioconcentration in fathead minnows of five organic chemicals
by Dale John Tischmak

A thesis submitted in partial fulfillment of the requirements for the degree Master of Science in Chemistry
Montana State University

Abstract:
A bioconcentration factor (BCF) in fathead minnows (Pimephales promelas) was determined for each of five chemicals in a series of 32-day tests. The chemicals were 1,2,4,5-tetrachloro-benzene (TCB), 2,4,6-trichloroanaline (TCA), pentachlorobenzene (PCB), propachlor, and chlordane. A 32-day BCF for each was again determined when all five chemicals were present simultaneously in a single test solution. In two additional tests, the BCFs for TCA and PCB were again determined when each was present at two to three times the test water concentrations for the initial tests.

A second test was also conducted on chlordane individually, but for a 64-day period.

The greatest difference between the BCFs determined individually and collectively was 44% for TCB. In no instance was the difference statistically significant, indicating that the presence of several chemicals in the test water did not affect individual BCFs. Increasing the test water concentrations of TCA and PCB did not affect their BCFs. Chlordane, unlike the other chemicals tested, did not reach saturation in the test fish prior to 32 days, but continued to bioaccumulate during a 64-day period.

Several experimental techniques were devised and evaluated, including a dosing system for introducing test chemicals into the test water.

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APPROVAL

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Dale John Tischmak

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date November 21, 1984

I don't want to bring a sour note
Remember this before you vote
We can all sink or we all float
'Cos we're all in the same big boat
One world is enough for all of us
One world is enough for all of us

From One World
by Sting

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ABSTRACT

A bioconcentration factor (BCF) in fathead minnows (Pimephales promelas) was determined for each of five chemicals in a series of 32-day tests. The chemicals were 1,2,4,5-tetrachlorobenzene (TCB), 2,4,6-trichloroanaline (TCA), pentachlorobenzene (PCB), propachlor, and chlordane. A 32-day BCF for each was again determined when all five chemicals were present simultaneously in a single test solution. In two additional tests, the BCFs for TCA and PCB were again determined when each was present at two to three times the test water concentrations for the initial tests. A second test was also conducted on chlordane individually, but for a 64-day period.

The greatest difference between the BCFs determined individually and collectively was 44% for TCB. In no instance was the difference statistically significant, indicating that the presence of several chemicals in the test water did not affect individual BCFs. Increasing the test water concentrations of TCA and PCB did not affect their BCFs. Chlordane, unlike the other chemicals tested, did not reach saturation in the test fish prior to 32 days, but continued to bioaccumulate during a 64-day period.

Several experimental techniques were devised and evaluated, including a dosing system for introducing test chemicals into the test water.

INTRODUCTION

Each year an ever-increasing number of synthetic chemicals is being marketed and applied to human needs. Many of these are potentially toxic chemicals, and their introduction into the natural environment [1, 2, 3, 4, 5, 6, 7, 8, 9] poses serious questions. What unintended negative consequences are or can be caused by the appearance of these chemicals in sensitive areas? The consequences to be considered include the results of introducing lethal doses of these chemicals, such as pesticides, to non-target animals. Also of importance are the subtle consequences to the biota of chronic sub-lethal exposure to hazardous chemicals. Such exposure may result in less visible effects such as shortened life, stunted growth, and impaired reproduction [10, 11].

It would be ideal to have definitive toxicity data for each newly developed chemical prior to marketing; the environmental hazards posed by each could then be carefully evaluated. Unfortunately there are too many new chemicals being introduced annually for present conventional toxicity screening systems to handle.

Through spills, disposal, and normal usage, lipophilic organic chemicals are nearly everywhere. Surface runoff can wash these chemicals from almost any location into lakes and streams.

Introduction through municipal wastewater is another source as is the chlorination of wastewater [12]. Direct application of the chemicals to natural waters is still another way of introducing them into the aquatic environment.

Once in an aquatic environment, the chemicals are available for uptake by a wide variety of organisms. Contamination of food sources by the chemicals followed by ingestion of the tainted food is one method by which aquatic animals can accumulate these chemicals. Being higher up in the aquatic food chain, however, does not necessarily mean that the particular species will accumulate more of the offending chemical [13]. Apparently only the largest aquatic predators will significantly biomagnify chemicals this way. Consumers such as humans could also be threatened.

A much more important mode of uptake for bioaccumulation by aquatic organisms is direct uptake of chemicals from the surrounding water [13, 14, 15, 16]. In the case of fishes, lipophilic chemicals can be directly acquired from water by transport through the gills or other membranes. This method, bioconcentration, accounts for much more of the total amount of chemical present in most fishes than does ingestion of contaminated food [13, 17].

Bioconcentration is the acquisition by an organism of a toxic chemical species to a point where the concentration of the chemical in the organism is greater than normally in the environmental source. The bioconcentration factor (BCF) is a

relative measure of this. The BCF for fishes is a proportionality constant determined by dividing the chemical concentration in the fish by the concentration in the water [24]. Food sources of the chemical are usually excluded. The BCF is somewhat time-dependent, so a constant value will be obtained only if a sufficient exposure period is allowed for a steady state to be reached. Bioconcentration factors also depend on the amount of lipid in the fish, so there will be some variation among fish species [18,19].

Bioconcentration data are difficult and expensive to obtain. To overcome these obstacles, several models relating chemical structure with biological activity have been developed to predict experimental results. For this study, the models of interest are those that predict bioconcentration factors.

The partition coefficient (P) has been recommended [20] as a link between chemical structures and bioconcentration factors by the equation

$$\log BCF = 0.542 \log P + 0.124$$

Equations of this type have been suggested by several researchers [21,22,23]. A modification of this equation by Veith et al. [24], that is valid for over six orders of magnitude of partition coefficients, has been reported as

$$\log BCF = 0.85 \log P - 0.70$$

The log P values can either be measured [25] or calculated [26,27].

The ultimate goal of these models is to provide data through structure-activity relationships without the necessity of actually

performing bioaccumulation tests. Though the models are not perfect in predicting every BCF from a known P, they do provide a correct estimate of the BCF that has significant value for most organic chemicals. The goal in structure-activity relationships is to predict BCFs with enough reliability to sort out only those chemicals which should be tested.

Even though the prediction models appear to be accurate, it is still important to compare BCF values obtained experimentally with those predicted theoretically. Expanding the experimental data base will indicate if any refinements are needed for the prediction method.

Conventional practice for obtaining BCF data has been to test chemicals separately on the animal species of interest. In many instances, however, this testing does not realistically represent environmental situations in that large amounts of a single given chemical are often not released separately nor are individually present in the environment. Mixtures of organic chemicals or complex effluents are frequently the case. Possible differences in a chemical's toxicity, caused by the presence of other competing or reinforcing chemicals, have not been studied in these laboratory tests.

As a group, organochlorine chemicals are very important both environmentally and economically. Many of the most effective synthetic pesticides, such as DDT, endrin, and 2,4,5-T, are in this group. This group also accounts for some of the organic chemicals with the most errant and deleterious environmental

distributions and deleterious environmental effects yet known [1, 10, 19]. These effects are due to the same chemical characteristics that make effective pesticides, namely high toxicity, environmental persistence, and food chain accumulation.

For this study, five organochlorine chemicals were selected for testing. They were: 2,4,6-trichloroaniline (TCA), 1,2,4,5-tetrachlorobenzene (TCB), pentachlorobenzene (PCB), 2-chloro-N-isopropylacetanilide (propachlor) and 1-exo,2-exo,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (chlordane). These chemicals covered both a range of log P values and a range of reported median lethal concentration (LC50) values (Table 1). TCA represented a low log P, TCB was an intermediate log P, and chlordane was a high log P chemical. Propachlor was included as a very low log P chemical, the presence of which might not be detected in the test animals. In addition to the selection of these four chemicals representing a spectrum of log P values, a fifth chemical, PCB, was selected because it had a similar log P and chemical structure to one of the others, TCB. This choice was made to see if the presence of two similar chemicals would affect the BCF of either.

Probable bioconcentration factors for each of the five chemicals were predicted using the method of Veith, et al. [24] (Table 2).

Table 1. Log P and 96-hr. LC50 values for the test chemicals to fathead minnows.

Chemical	Log P	96-hr. LC50, ug/l
Propachlor	2.75 [25]	490 [29]
TCA	3.80 [27]	1,000-10,000[a] [12]
TCB	4.67 [37]	1,070 [3]
PCB	5.19 [37]	250 [38]
Chlordane	6.00 [24]	37 [5]

[a]96-hour median tolerance limit from a static bioassay.

Table 2. Calculated bioconcentration factors.

Chemical	Log BCF	BCF
Propachlor	1.64	43.7
TCA	2.53	339
TCB	3.27	1,860
PCB	3.71	5,130
Chlordane	4.40	25,100

There were additional reasons for selecting these five chemicals. It has been shown that these chemicals are present in the environment [2,3,12,29,30]. Together they were analytically

acceptable in that all responded and separated nicely in the available gas chromatograph.

The primary objective of this study was to determine individual chronic bioconcentration factors for each of the five chemicals mentioned through separate 32-day tests on fathead minnows (Pimephales promelas), and to compare these with individually determined BCFs for each chemical when all five were simultaneously present in the test water. A secondary objective was to observe how the water concentration of test chemicals might affect BCFs. That is, does a constant factor for proportionality (BCF) exist? The tertiary objective of this study was to determine a maximum BCF for chlordane, and whether the 32-day BCF was an adequate measure of its bioaccumulation potential.

MATERIALS AND METHODS

Exposure System

Several flow-through bioconcentration tests were performed on fathead minnows. Modifications were made to a basic diluter design [31] to devise an apparatus that delivered a constant test chemical concentration to a single tank of test organisms.

Bozeman city water, dechlorinated by a Culligan® water dechlorinater, was fed into a 38-liter plastic tub that served as a headbox for the system. A heater kept the water at a constant temperature. Water drained from this tub through a solenoid valve into a 4-liter rectangular glass box. A siphon tube on the side of this box connected to a float switch chamber which served to open and close the solenoid valve. The float switch chamber was equipped with a siphon tube that drained into another 4-liter glass box used as a mixing chamber. This portion of the diluter system delivered 960 ml of water to the mixing chamber every 6.5 minutes.

The test chemical was conveyed to the mixing chamber by a peristaltic pump that delivered 4 ml/minute. The diluter stock chemical solutions were made using dechlorinated water and concentrated solutions of the test chemicals in acetone. The chemical solutions were mixed in 4-liter soft glass bottles; brown bottles were used for this purpose to reduce the amount of

photodecomposition. The chemical solutions were pumped directly from these brown bottles to the mixing chamber.

Water draining from above into the mixing chamber with the test chemical served to mix the solution. As the mixing chamber filled, the test water would flow from the mixing chamber through a siphon tube to the test aquarium. The test aquarium was a 20-liter glass aquarium holding 12 liters of test water. The aquarium was covered with aluminum foil to reduce the loss of chemicals resulting from volatilization. The aquarium volume was replaced approximately once every 90 minutes. The aquarium effluent was passed through an activated carbon filter before being discharged. The discharge was monitored for presence of the test chemicals.

The stock chemical solutions for the dilutor were made by adding an appropriate amount of the acetone solutions to 3 liters of water in a brown bottle and stirring with a magnetic stir bar. The final liter of water was then added and stirred. The amount of acetone solution added was adjusted until the desired test water concentration of the test chemical was obtained. The anticipated water concentrations were a few micrograms per liter. The amounts and concentrations of the acetone solutions used are summarized in Table 3. For the final test where all five chemicals were present in the test water, a single acetone solution with all the chemicals was used.

A control aquarium was supplied with water directly from the headbox via a polyethylene siphon tube. The flow rate was 50 ml/min.

Table 3. Parameters for making four liters of diluter stock solutions.

Test Chemical	Concentration in Acetone, mg/l	Volume Acetone Solution Added, ml	Calculated Concentration, ug/l
TCB (Test 1)	100	15.0	375
TCA (Test 1)	200	6.0	300
PCB (Test 1)	1010	1.6	404
Propachlor	2000	2.0	1000
Chlordane (Test 1)	1020	0.3	76.5
Chlordane (Test 2)	184	1.9	87.4
Mixture		1.1	
TCB	2720		748
TCA	1490		410
Chlordane	418		115
PCB	2730		751
Propachlor	6420		1770

Test Organisms

The test fish were fathead minnows obtained from Fattig Fish Farm (Brady, Nebraska) as 3-month-old fry. Tests were conducted over an 8-month period, beginning when the fish were 4 months old.

During culture prior to testing, the fish were exposed 3-4 times per month to a 3% formalin solution to reduce external parasites. Fish for a given test were acclimated to the test dilution water (Table 4) for 3-5 days. The fish were fed ad libitum three times per week during the acclimation and test periods.

Test Methods

Five to 7 days before each test was started, the diluter system was activated so as to obtain a stable test chemical concentration in the test chamber. On the starting day of each test, the test chemical concentration in the test water was determined before any fish were added. Fish were then randomly selected from the control aquarium and put into the test chamber.

Once a week the test fish were transferred to a clean aquarium filled with test water. The siphon tube from the mixing chamber was moved to the clean aquarium about two hours prior to transferral. In the meantime, an air stone bubbler kept the original aquarium oxygenated.

Test water samples were analyzed five times a week for test chemical concentration. Unless otherwise noted, fish were sampled after 0, 1, 4, 8, 16, and 32 days of exposure. To determine both the maximum chlordane BCF and whether a 32-day period was adequate to reach steady-state BCF, three fish were sampled each week over nine weeks of exposure (Chlordane Test 2).

Table 4. Chemical characteristics of dilution water in B-1 Cooley Laboratory.

Chemical	Concentration (mg/L)
Al	<0.02
B	<0.01
Ba	0.043
Be	<0.002
Ca	19.3
Cd	<0.005
Cr	<0.1
Cu	<0.006
K	1.27
Mg	6.2
Mn	<0.002
Mo	<0.05
Na	2.6
Ni	<0.05
P	<1
Pb	<0.01
Si	5.6
Sr	<0.05

To determine how the test chemical water concentration affected bioconcentration, two individual chemical tests were repeated at increased toxicant concentrations (TCA Test 2 and PCB Test 2).

Analysis of test water for dissolved oxygen, pH, temperature, and conductivity was performed three times per week. Free and residual chlorine was determined once a week. Total alkalinity, total hardness, calcium and magnesium were determined twice per test.

Water Chemistry

Several water variables were monitored throughout the tests (Table 5). The pH was measured with a Beckman pHasar-I pH meter. Conductivity was measured with a Yellow Springs Instruments Model 33 SCT meter.

Test Chemicals

The five test chemicals were obtained from Chem Service (West Chester, PA) in small quantities at 95% or higher purity. The chlordane used was the purified alpha-isomer.

Reagents and Materials

All organic solvents used were of the pesticide (glass distilled) grade. Glass chromatography columns (11 x 300 mm) with removable stopcocks were prepared by first inserting a glass wool plug at the end of each column. On top of this was placed 12 cm

of Florisil covered by 2.5 cm of sodium sulfate. The glass wool was solvent-extractd for 12 hours in a soxhlet extractor. The

Table 5. Water chemistry parameters and analytical methods.

Parameter	Method
pH	EPA No. 150-1[a]
Free & Residual Chlorine	APHA No. 114C[b]
Dissolved Oxygen	APHA No. 421B[c]
Conductivity	APHA No. 205[c]
Temperature	APHA No. 212[c]
Total Alkalinity	APHA No. 403[c]
Total Hardness	APHA No. 314B[c]
Calcium	APHA No. 311C[c]
Magnesium	APHA No. 318C[c]
Nitrate	APHA No. 418A[c]
Nitrite	APHA No. 419[c]
Ammonia	APHA No. 417B[c]

[a]U.S. Environmental Protection Agency [32].

[b]American Public Health Association, et al. [33].

[c]American Public Health Association, et al. [34].

Florisil was PR grade 60/100 mesh. The sodium sulfate was reagent grade that had been heated in a muffle furnace at 400°C for 12 hours. The Florisil columns were stored at 130°C for at least 12 hours prior to use.

Gas Chromatograph

Analyses for test chemicals both in water samples and from fish tissues were performed on a Varian Vista 6000 gas chromatograph with a CDS 401 terminal and a Model 8000 autosampler. A ^{63}Ni electron capture detector (ECD) was used in conjunction with a 15 m x 0.25 mm (I.D.) fused silica capillary column that was Durabond coated with DB-5 (J&W Scientific, Inc., Rancho Cordova, CA). The carrier gas was ultra-high purity helium and the ECD make-up gas was ultra-high purity nitrogen. The column head pressure was 15 psi. The make-up flow was 20 ml/min and the auxiliary flow was 10 ml/min. The split flow was 50 ml/min on 3 ul injections. Two injections per sample were made and averaged. The variability between injections never exceeded 6%.

Analyses for individual chemical tests were done with isothermal oven temperatures. When the five chemicals were mixed the analyses were done with an oven temperature program (Table 6).

Water Analysis

The test chemical concentrations in the test water were monitored by gas chromatographic analyses of test water extracts. The extracts were obtained by placing 50 ml of the test water into a 100 ml volumetric flask containing 5 ml of n-heptane. A teflon-coated magnetic stir bar was added and the flask contents were vigorously stirred for one hour. After stirring, the flasks were set aside for 15-30 minutes to allow the phases to separate. Double distilled water (Corning Model 2900 glass still) was then

Table 6. Gas chromatograph oven conditions.

Test Chemical	Oven Conditions
TCB	140°C isothermal for 6 min.
TCA	165°C isothermal for 4 min.
Chlordane	215°C isothermal for 10.2 min.
PCB	155°C isothermal for 7.4 min.
Propachlor	175°C isothermal for 6 min.
Mixture	140°C isothermal for 3 min., 4°C/min. to 185°C, 0.5 min. hold, 20°C/min. to 225°C, 6.75 min. hold

added to the flask to bring the heptane into the neck of the flask. An aliquot of the heptane was placed in an autosampler vial and analyzed. This extraction method gave a ten-fold concentration increase of the toxicant in the heptane as compared to in the water. This was compensated for in the analyses.

The recovery rate of each chemical from water with all five chemicals present was comparable to the extraction efficiency of separate water spikes (Table 7). Water spikes were made by diluting the desired amount of test chemical in acetone to 1 liter with dechlorinated water and mixing.

The variability of a particular result in a given test was identified by the relative standard deviation (RSD). The RSD was the percentage of the result's mean in the standard deviation of all the measurements made during the test.

Table 7. Recovery efficiency from spiked water samples.

Test	Chemical	Percent Recovery (Relative Standard Deviation)	No. Spikes Analyzed
TCB Test 1	TCB	103 (3%)	26
TCA Test 1	TCA	97 (3%)	11
Chlordane Test 1	Chlordane	102 (6%)	8
PCB Test 1	PCB	86 (4%)	10
Propachlor		57 (3%)	12
Mixture			5
	TCB	97 (7%)	
	TCA	99 (1%)	
	PCB	78 (9%)	
	Propachlor	56 (4%)	
	Chlordane	98 (4%)	

Fish Tissue Analysis

The test chemical concentration in each fish was determined by gas chromatographic analysis of tissue extracts of each fish. The method described below is similar to that of Benville and Tindle [35].

On the days fish were to be sampled, fish were removed from the test aquarium, blotted dry and weighed. An amount of sodium

sulfate, approximately four times the mass of the fish sampled, was added to the container holding the fish. The fish were then frozen with dry ice. After the fish were thoroughly frozen, each fish was homogenized in a 200-ml stainless steel blender cup that had been cooled by mixing dry ice in the blender cup. The use of dry ice reduced the amount of material that adhered to the sides of the cup. A double layer of aluminum foil held in place by the blender cup cap served as the lid.

The fish homogenate was transferred, using a powder funnel cooled in dry ice, to a 35 x 90 mm glass extraction thimble with a 40 micron fritted glass bottom. The blender cup was rinsed after homogenating each fish with 10 ml of hexane. The rinse hexane was placed in a 125-ml soxhlet flask.

After the thimble and contents had reached room temperature, the thimble was placed in a 125-ml soxhlet extraction apparatus and extracted with 100 ml of hexane, including the rinse hexane, for four hours [41]. Following extraction the hexane extracts were concentrated using Kuderna-Danish concentrators to a volume of about 10 ml. This concentrated extract was purified by chromatographic separation on Florisil columns. The concentrated extracts were placed on columns that had been wetted with 5% (v/v) methyl-t-butyl ether in heptane. The Florisil columns were eluted with 50 ml of the 5% solution. The eluants were collected in 100 ml volumetric flasks that were brought up to volume with heptane after the elution was complete. These solutions were then

analyzed by gas chromatography to determine the test chemical concentrations in the fish tissues.

As a validity test for the hexane extraction method, several test fish from each test were extracted with a 1:1 (v/v) methylene chloride in hexane solvent mixture concurrent with the usual hexane extractions. No differences in the determined BCFs for the two solvents were observed. Hexane alone was an adequate extraction solvent for fish tissue in this study.

When analyzing for propachlor, additional elution steps had to be taken to recover the propachlor. After elution with the 5% methyl-t-butyl ether in heptane, the column was eluted with 50 ml of 20% (v/v) ethyl ether in petroleum ether to remove most of the fish lipids [36]. This eluant was discarded. All of the propachlor was eluted in the next eluant volume, which volume was 50 ml of 5% (v/v) acetone in heptane.

Samples to determine recovery of chemicals from fish tissue were made by injecting intraperitoneally up to 10 ul of an appropriate acetone solution into control fish (Table 8).

Lipid Determination

The lipid percentage of at least one fish from each sample group was determined. The hexane extract from each fish for which the percent lipid was to be determined was reduced in volume to 20 ml. Of this, 2 ml was placed in an aluminum weighing pan that had been rinsed with acetone, baked at 100°C for two hours, cooled in a dessicator, and then re-weighed. The hexane was allowed to

evaporate from the pan. The pan was then baked for 15 minutes at 100°C, cooled in a dessicator, and weighed. The increase in mass of the pan was attributed to the fish lipids. This mass was corrected for the total extract volume and divided by the mass of the fish to determine the percentage of lipid in the fish. The final test chemical concentration in the fish involved with this determination was similarly corrected.

Table 8. Recovery efficiency from spiked fish samples.

Test	Chemical	Percent Recovery (Relative Standard Deviation)	No. Fish Spikes Analyzed
TCB Test 1	TCB	99 (6%)	12
TCA Test 1	TCA	90 (5%)	11
Chlordane Test 1	Chlordane	106 (5%)	3
PCB Test 1	PCB	97 (6%)	6
Propachlor	Propachlor	45 (11%)	13
Mixture			4
	TCB	98 (4%)	
	TCA	93 (5%)	
	Chlordane	102 (4%)	
	PCB	96 (5%)	
	Propachlor	49 (8%)	

RESULTS

Test Water Concentrations

The mean of the test water concentrations of the various test chemicals over the durations of the tests are reported in Table 9. The desired water concentrations for each chemical were obtained, and concentrations units were converted from ug/l to ug/g prior to determining bioconcentration factors.

Test Water Chemistry

The water chemistry variables for each test are reported in Tables 10 through 15. The water variables for PCB Test 2 and TCB Test 2 correspond to the propachlor test water variables (Table 14) because these tests were conducted during the same time period. Similarly, the water variables for Chlordane Test 2 correspond to the mixed chemical test water variables (Table 15).

Lipid Content

The percentage of lipid in fish from each test was determined (Table 16). In addition, lipid content of fish extracted with hexane was compared with lipid content determined for fish extracted with a 1:1 (v/v) mixture of hexane and methylene chloride. No significant differences were found between the lipid percentages, so hexane was a valid extraction solvent.

Table 9. Concentrations of the test chemicals in test water.

Test	Chemical	Average Concentration, ug/l	RSD, %	No. Samples
TCB Test 1	TCB	1.40	16	24
TCA Test 1	TCA	2.17	13	27
Chlordane Test 1	Chlordane	0.88	16	36
PCB Test 1	PCB	1.87	8.6	25
Propachlor	Propachlor	29.1	23	25
PCB Test 2	PCB	4.62	13	10
TCA Test 2	TCA	5.87	2.9	7
Chlordane Test 2	Chlordane	0.89	14	44
Mixture				23
	TCB	3.82	16	
	TCA	7.19	10	
	PCB	3.93	18	
	Propachlor	49.3	21	
	Chlordane	1.03	21	

Table 10. Water chemistry variables from TCB Test 1.

Parameter	Mean	RSD, %	Range	No. of Measurements
Dissolved Oxygen (mg/l)	6.0	10	5.0-7.0	17
Conductivity (umhos/cm)	158	5	152-174	14
pH	7.40	2	7.20-7.60	4
Temperature (°C)	19.9	4	19.1-21.6	14
Chlorine (ppm)	<0.02	-	-	5
Alkalinity (mg/l as $CaCO_3$)	74	1	74-75	2
Hardness (mg/l as $CaCO_3$)	80	1	80-81	2
Calcium (mg/l)	25	-	-	2
Magnesium (mg/l)	4.2	-	-	2

Table 11. Water chemistry variables from TCA Test 1.

Parameter	Mean	RSD, %	Range	No. of Measurements
Dissolved Oxygen (mg/l)	6.3	15	3.3-7.0	17
Conductivity (umhos/cm)	143	4.3	134-152	14
pH	7.63	1.8	7.47-7.88	14
Temperature (°C)	20.9	2.0	20.3-21.9	14
Chlorine (ppm)	<0.02	-	-	5
Alkalinity (mg/l as $CaCO_3$)	91	35	68-114	2
Hardness (mg/l as $CaCO_3$)	76	11	70-82	2
Calcium (mg/l)	30	31	23-36	2
Magnesium (mg/l)	7.2	-	3.3-11	2

Table 12. Water chemistry variables from Chlordane Test 1.

Parameter	Mean	RSD, %	Range	No. of Measurements
Dissolved Oxygen (mg/l)	8.9	4.2	8.2-9.7	18
Conductivity (umhos/cm)	152	6.7	135-169	18
pH	7.64	1.2	7.51-7.93	18
Temperature (°C)	13.2	4.8	11.6-14.3	18
Chlorine (ppm)	<0.02	-	-	6
Alkalinity (mg/l as $CaCO_3$)	82	3.4	80-84	2
Hardness (mg/l as $CaCO_3$)	88	3.2	86-90	2
Calcium (mg/l)	25	10	24-27	2
Magnesium (mg/l)	6.0	14	5.4-6.6	2

Table 13. Water chemistry variables from PCB Test 1.

Parameter	Mean	RSD, %	Range	No. of Measurements
Dissolved Oxygen (mg/l)	8.5	3.1	8.1-8.9	12
Conductivity (umhos/cm)	151	4.9	137-165	12
pH	7.62	1.1	7.49-7.85	12
Temperature (°C)	13.6	4.6	12.6-14.4	12
Chlorine (ppm)	<0.02	-	-	5
Alkalinity (mg/l as $CaCO_3$)	85	1.7	84-86	2
Hardness (mg/l as $CaCO_3$)	92	3.1	90-94	2
Calcium (mg/l)	27	-	-	2
Magnesium (mg/l)	5.8	11	5.4-6.3	2

Table 14. Water chemistry variables from propachlor test.

Parameter	Mean	RSD, %	Range	No. of Measurements
Dissolved Oxygen (mg/l)	7.2	12	5.6-8.7	11
Conductivity (umhos/cm)	154	5.7	137-169	11
pH	7.42	2.6	7.05-7.65	11
Temperature (°C)	16.0	9.8	13.9-18.3	11
Chlorine (ppm)	<0.02	-	-	5
Alkalinity (mg/l as $CaCO_3$)	89	4.8	86-92	2
Hardness (mg/l as $CaCO_3$)	96	2.9	94-98	2
Calcium (mg/l)	30	16	27-34	2
Magnesium (mg/l)	4.6	50	3.0-6.3	2

Table 15. Water chemistry variables from chemical mixture test.

Parameter	Mean	RSD, %	Range	No. of Measurements
Dissolved Oxygen (mg/l)	5.6	14	3.9-6.8	14
Conductivity (umhos/cm)	169	28	128-269	14
pH	7.42	1.0	7.32-7.55	14
Temperature (°C)	19.1	6.2	17.9-21.8	14
Chlorine (ppm)	<0.02	-	-	5
Alkalinity (mg/l as $CaCO_3$)	62	-	-	2
Hardness (mg/l as $CaCO_3$)	74	11	68-80	2
Calcium (mg/l)	20	6.3	19-21	2
Magnesium (mg/l)	5.8	23	4.9-6.8	2

Table 16. Percentage of lipid in test fish.

Test	Average % Lipid	RSD, %	Measurements	Average Test Fish Mass, g
TCB Test 1	7.1	21	7	0.78
TCA Test 1	10	24	6	1.42
Chlordane Test 1	10	15	8	2.21
PCB Test 1	8.3	13	5	2.22
Propachlor	7.4	11	4	1.87
PCB Test 2	6.8	19	2	1.38
TCA Test 2	9.7	33	3	2.72
Mixture	8.9	45	6	2.49
Chlordane Test 2	8.3	38	5	2.05

Bioconcentration Factor Determinations

The BCF for each fish was determined by dividing the concentration of the chemical in the fish by the averaged water concentration of the chemical up to the sampling date of the fish. Both measurements had units of ug/g. The results from the individual chemical BCF tests were then compared with the results from the chemical mixture BCF test to see if the results were significantly different.

Of the five chemicals tested, propachlor gave the lowest BCF results. Propachlor did not measurably bioconcentrate in any of the fish tested, so no data are included for it. Propachlor is

probably more easily metabolized by fish than the other four chemicals [29,40] and thus eliminated rather readily.

If propachlor had bioconcentrated as expected, the analytical methods used should have detected it. Because propachlor is rather polar and the BCF should have been quite low, gel permeation chromatography may have been a more effective method of analysis and may have given a measurable BCF [39].

From BCF data obtained for TCB from TCB Test 1 and the mixed chemical test, the peak BCF for both was reached on Day 8 (Table 17 and Figure 1). The difference between the two 32-day BCF values is not statistically significant.

Table 17. TCB bioconcentration factors.

Test	Exposure Period, days	BCF	RSD, %	No. Fish Analyzed
TCB Test 1	0	0	-	5
	1	1760	18	5
	2	2660	13	5
	4	3760	5.0	5
	8	6190	7.0	5
	16	4950	30	5
	32	3990	24	5
Mixture	0	0	-	3
	1	1880	5.1	4
	4	5620	13	4
	8	6700	14	4
	16	6610	14	4
	32	7100	34	8

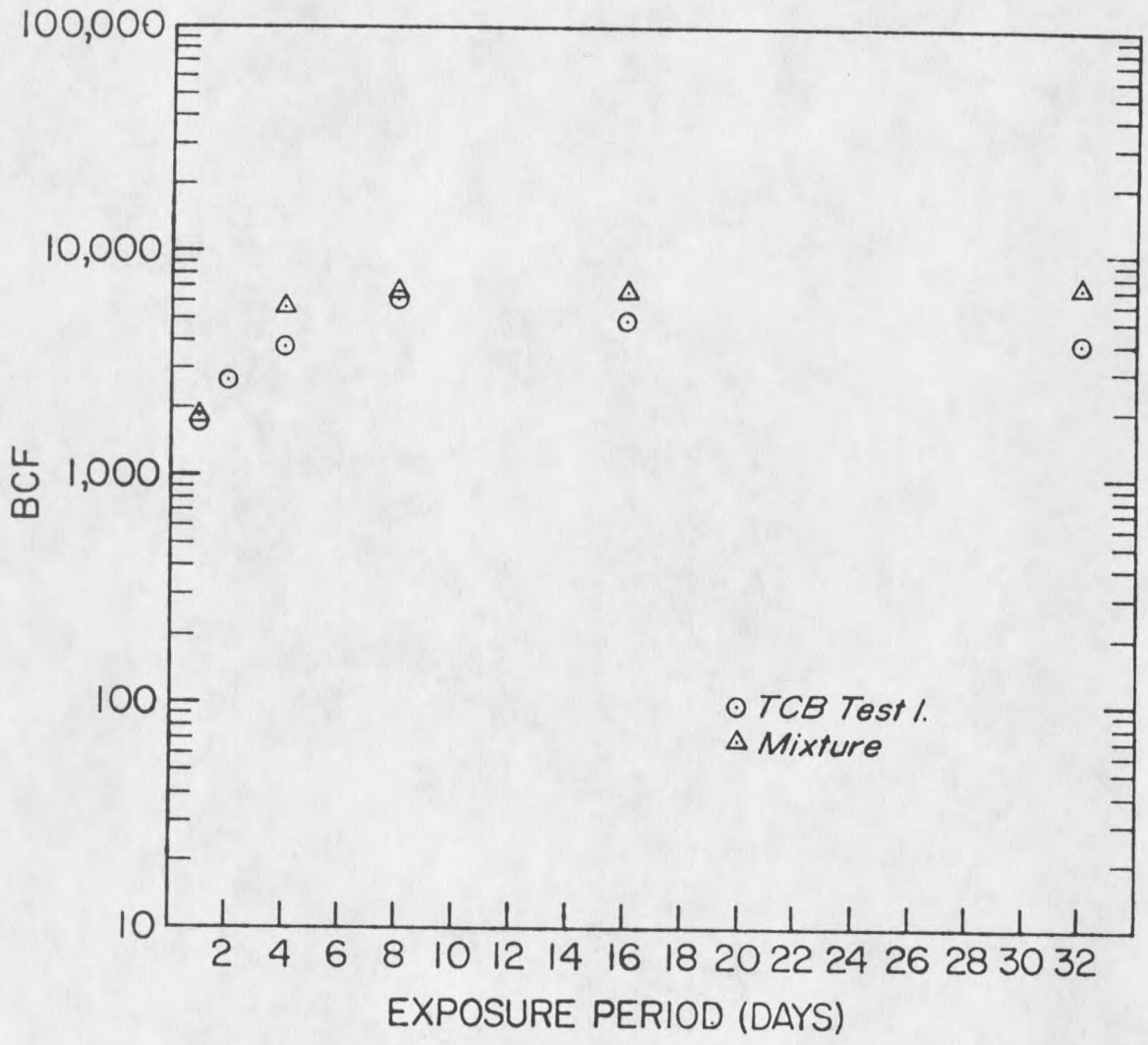

Figure 1. TCB bioconcentration profiles.

In the tests monitoring TCA, the maximum BCF was at least approached if not substantially reached by Day 2. The maximum BCF was always reached by Day 8 (Table 18 and Figure 2).

Table 18. TCA bioconcentration factors

Test	Exposure Period, days	BCF	RSD, %	No. Fish Analyzed
TCA Test 1	0	0	-	3
	2 hr.	99	10	5
	4 hr.	191	22	5
	1	664	12	5
	2	737	12	5
	4	727	16	5
	8	892	10	5
	16	680	13	5
	32	531	26	5
TCA Test 2	0	0	-	2
	2	623	28	3
	4	555	34	3
	7	606	26	3
Mixture	0	0	-	3
	1	533	11	4
	4	812	15	4
	8	657	17	4
	16	576	15	4
	32	625	31	8

TCA was taken up and the BCF stabilized more quickly than any of the other chemicals tested. The uptake profiles of all three TCA tests were very similar. There was no significant difference between the two 32-day BCF values.

PCB Test 1 and PCB Test 2 had very similar uptake profiles (Table 19 and Figure 3). PCB uptake in the chemical mixture test

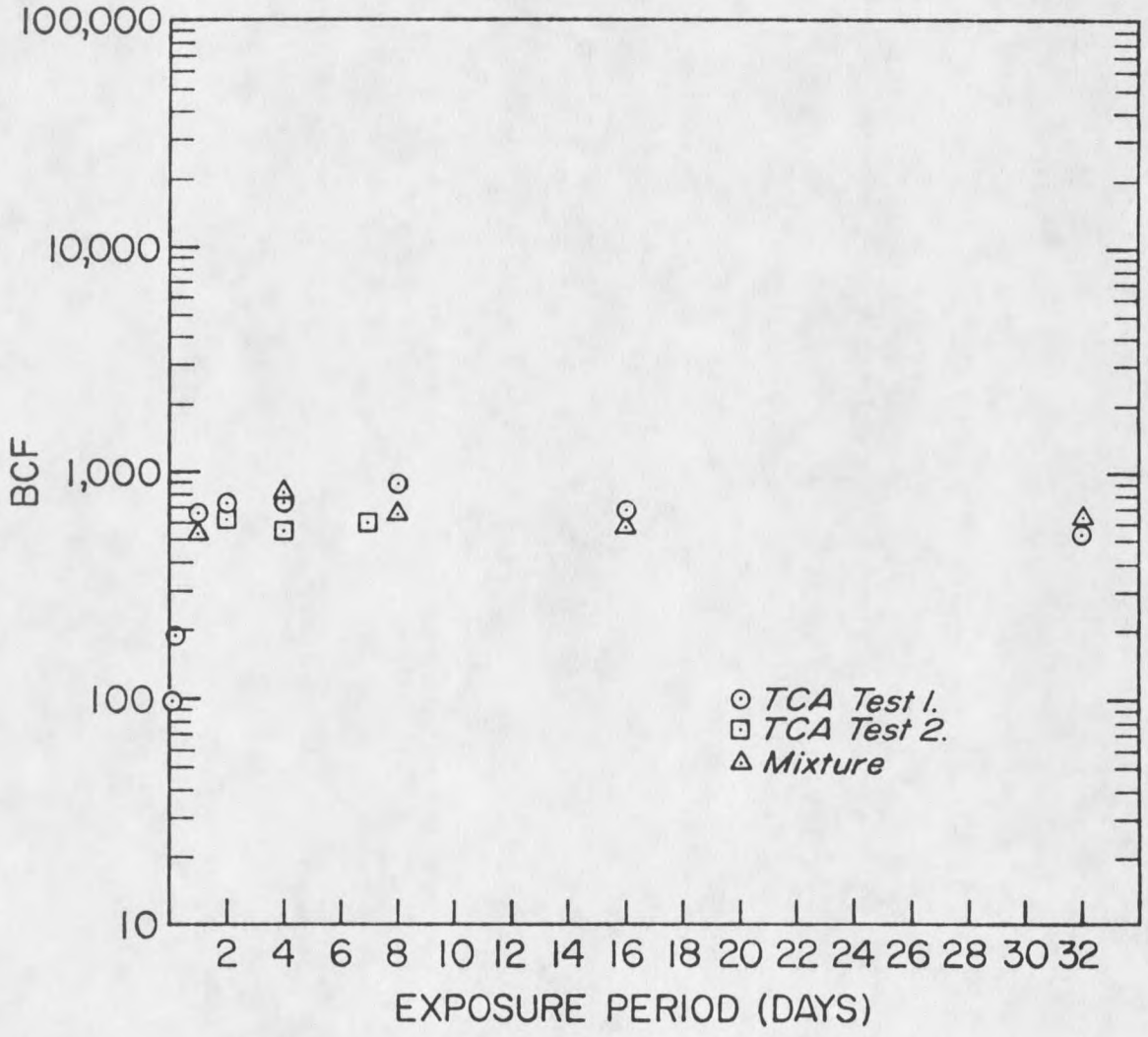

Figure 2. TCA bioconcentration profiles.

Table 19. PCB bioconcentration factors.

Test	Exposure Period, days	BCF	RSD, %	No. Fish Analyzed
PCB Test 1	0	0	-	2
	1	974	16	5
	4	4840	16	4
	8	9750	17	3
	16	10,100	11	3
	32	16,900	11	4
PCB Test 2	0	0	-	2
	7	6410	8.9	3
	8	8500	8.1	3
	11	9530	8.0	3
Mixture	0	0	-	3
	1	2310	11	4
	4	7810	11	4
	8	12,700	22	4
	16	16,200	2.0	4
	32	19,700	31	8