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TOXAPHENE: CHRONIC TOXICITY TO FATHEAD MINNOWS AND CHANNEL CATFISH

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
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TOXAPHENE: CHRONIC TOXICITY TO FATHEAD MINNOWS AND CHANNEL CATFISH

by

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FOREWORD

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

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

--to assess the effects of ecosystems on pollutants

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

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

This report studies the effects of the insecticide toxaphene on two species of fish, fathead minnows and channel catfish, when continuously exposed to five different toxaphene concentrations for periods of 8 to 10 months.

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ABSTRACT

Fathead minnows (Pimephales promelas) and channel catfish (Ictalurus punctatus) were continuously exposed to several toxaphene concentrations (13-630 ng/l) in flow-through diluter systems for 8 to 10 months. Growth and backbone quality of adult fathead minnows were decreased at 97 and 173 ng/l exposures, but adult channel catfish were not affected by toxaphene. Effects on reproduction were observed only in channel catfish in the 630 ng/l concentration: the period from pairing to spawning was increased and the amount of gelatinous matrix surrounding the eggs was reduced. Survival of fathead minnows was not affected by toxaphene, but the no-effect concentration for fry growth and bone quality was below 54 and 97 ng/l, respectively. Channel catfish fry survival and growth were reduced in the 299 and 630 ng/l exposures, and bone quality was altered in concentrations as low as 72 ng/l. The maximum toxaphene accumulation from water to fish was 69,000 times in fathead minnows and 50,000 times in channel catfish. Toxaphene was excreted very slowly in both species.

This report was submitted in partial fulfillment of Contract No. EPA-IAG-141(D) by the Fish-Pesticide Research Laboratory, Fish and Wildlife Service (USDI) under sponsorship of the U.S. Environmental Protection Agency. Work was completed in May 1976.

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ACKNOWLEDGMENTS

Technical assistance and maintenance were provided by D. R. Buckler, W. A. McAllister, and D. C. Zumwalt. Collagen, hydroxyproline, calcium, and phosphorus assays were conducted by Sandra Cummins, Linda Fryer and S. J. Hamilton and toxaphene residue analyses by J. L. Johnson. The backbone illustrations were drawn by Becky Turk. Mayo Martin and Harry Dupree gave assistance and offered suggestions in the channel catfish study and Leonard Mueller assisted us throughout the project. Portions of the study were funded by the Fish and Wildlife Service, United States Department of the Interior.

SECTION 1

INTRODUCTION

Toxaphene, an organochlorine insecticide, has been extensively used in the United States for over 20 years to control agronomic insect pests--particularly in the Lower Mississippi Valley, one of the most intensively farmed areas in the United States, where it is used for control of cotton insect pests. A pesticide use survey[1] of the Lower Mississippi Valley and Gulf Coastal Prairie showed that toxaphene was being applied 8 to 12 times during July through September at a rate of 2.2 to 3.4 kg/ha per application.

The distribution of toxaphene in fish, as found by the 1973 and 1974 National Pesticide Monitoring Program[2], indicated that as an aquatic contaminant, the insecticide is mainly limited to the Southeastern United States (Fig. 1). However, isolated occurrences of toxaphene residues were found in rainbow trout (Salmo gairdneri) from the Kenai River, Alaska, in 1973 and in largemouth bass (Micropterus salmoides) from the Colorado River, Arizona, in 1974. Residues in fish ranged from non-detectable (<0.05 ug/g) to 51 ug/g overall, and of the species containing toxaphene in the important cyprinid (minnow) and ictalurid (catfish) families, the concentrations ranged from 0.5 to 38 and 0.5 to 51 ug/g, respectively. Mayer et al.[3] found that whole body residues of toxaphene of 0.6 ug/g in fry of brook trout (Salvelinus fontinalis) were relatable to detrimental effects.

The acute effects of toxaphene on aquatic organisms and its persistence in the aquatic environment have been summarized[3], but data describing the chronic effects in fish species other than brook trout, especially those important in the Southeastern United States, are absent. Fathead minnows (Pimephales promelas) and channel catfish (Ictalurus punctatus) are indigenous to freshwater systems east of the Rocky Mountains and are of high economic significance in the areas most contaminated with toxaphene. The fathead minnow is an important forage and commercial baitfish, and poisoning of minnows by toxaphene and endrin in Arkansas and Louisiana has been reported[4]. The widespread use of channel catfish by sportsmen, commercial fishermen, and fish farmers makes this species an important natural resource. Because of the continued heavy use of toxaphene in the Southeastern United States, its presence in indigenous fish species, and the importance of fathead minnows and channel catfish, we undertook this study to determine the effects of toxaphene on these two species.

The purpose of the research was to develop water quality criteria for toxaphene which will assist the Fish and Wildlife Service and the Environmental Protection Agency to better manage and protect fishery resources.

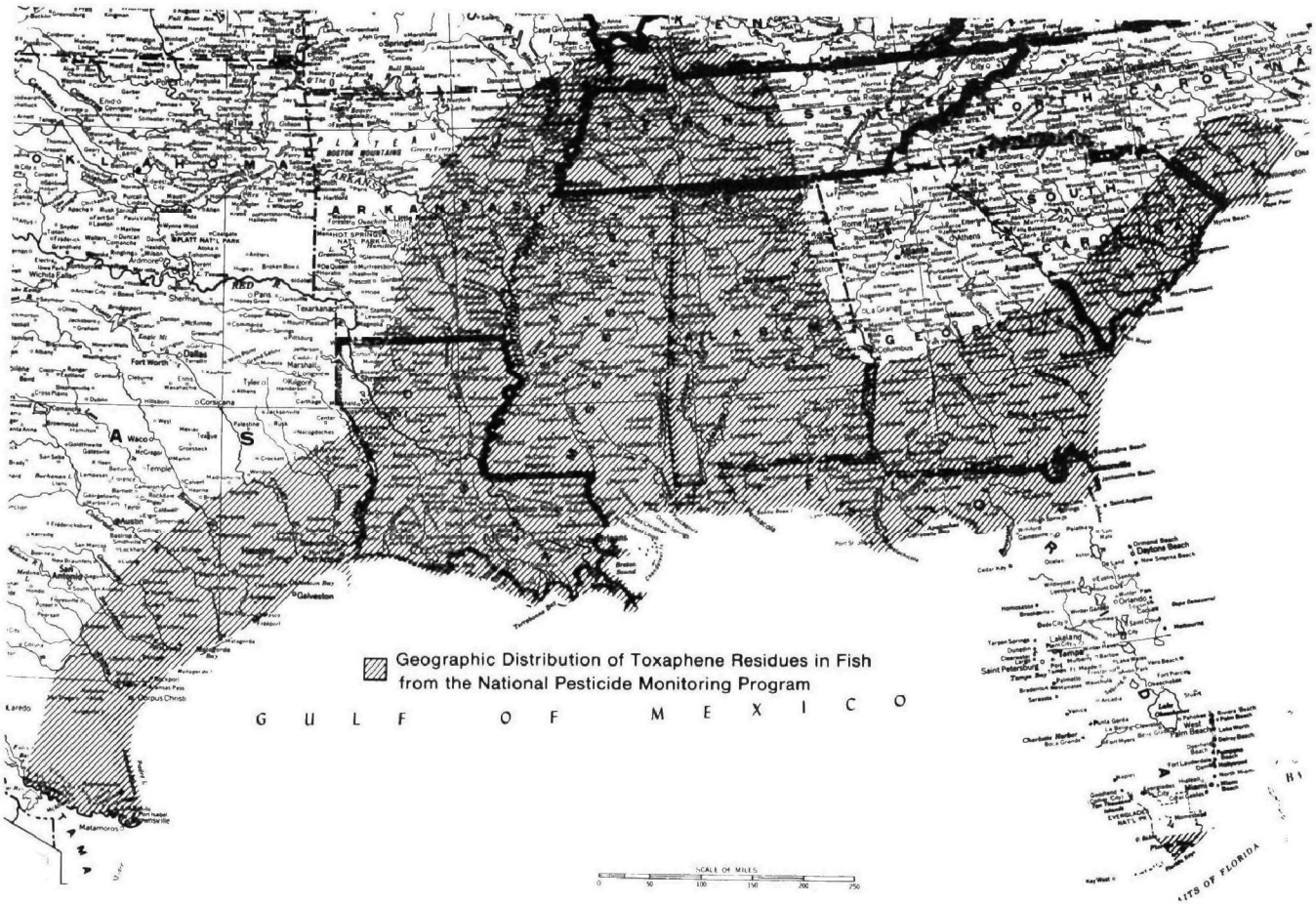


Figure 1. Geographic distribution of fish containing detectable toxaphene residues in the 1973 and 1974 National Pesticide Monitoring Program.

Specific objectives of the study were: (1) to develop laboratory techniques for testing channel catfish through their reproductive cycle in chronic toxicity tests; (2) to determine the effect of continuous exposures of toxaphene on the growth, reproduction, and mortality in fathead minnows and channel catfish; (3) to further evaluate the potential use of collagen and hydroxyproline as indicators or predictors of abnormal growth and development in fish; and (4) to determine the degree of accumulation and elimination of toxaphene in fathead minnows and channel catfish.

SECTION 2

CONCLUSIONS

On the basis of growth, reproduction, mortality, and bone development, the maximum acceptable concentration of toxaphene in water was between 25 and 54 ng/l for fathead minnows and between 49 and 72 ng/l for channel catfish.

Toxaphene was accumulated 10,000 to 69,000 times water concentrations by various life stages of fathead minnows and 17,000 to 50,000 times by channel catfish. Excretion of toxaphene was very slow in fathead minnows and channel catfish, requiring up to 56 days for 36% elimination.

Collagen and hydroxyproline are sensitive indicators of growth and development in fathead minnows and channel catfish.

Except for their large space requirements, channel catfish are highly desirable test organisms for use in chronic toxicity tests.

SECTION 3

RECOMMENDATIONS

For the protection of fathead minnows and channel catfish, toxaphene concentrations in water should be less than 54 and 72 ng/l, respectively.

Laboratory tests show that collagen and hydroxyproline are good biochemical indicators or predictors of growth and development in fishes and should be evaluated for applicability to field monitoring of biological effects.

Channel catfish should be included among species recommended for chronic toxicity tests, especially where there is a possibility of contamination of water by a pesticide or other pollutant in the Southeastern United States.

SECTION 4

MATERIALS AND METHODS

GROWTH, REPRODUCTION, AND MORTALITY

General

Seven-day-old fathead minnows obtained from the National Water Quality Laboratory, Duluth, Minnesota, were held in proportional diluter systems at 25 C until the tests were initiated at 40 days of age. The fish were fed a commercial trout starter (EWOS) ad libitum, which was supplemented daily with live brine shrimp nauplii for the first 30 days. Adult channel catfish were purchased from Schroeder's Fish Farm, Carlisle, Arkansas. Total body residues of toxaphene averaged 1.2 ug/g, which was the lowest residue found in analysis of fish from several sources. The catfish were held in raceways at 16 C and fed a maintenance diet until the tests were started[5]. The adult fish were fed a floating commercial catfish food (Purina); fry were fed the Modified Oregon Test Diet[6] ad libitum. Eggs collected from both species during the test were treated for 3 minutes with 60 mg/l of malachite green for the first 3 days. Well water (characteristics previously described[3]) was passed through an ultraviolet sterilizer system before it entered the diluter systems.

Diluter systems[7] with the modification of McAllister et al.[8] and a dilution factor of 0.5 between the concentrations were used to deliver five concentrations of toxaphene and a control for the chronic tests. We attempted to select a concentration range that included a no-effect concentration, which was estimated by multiplying the lethal threshold concentration (determined when the rate of death was 10% or less of the original number of fish in any concentration during the preceding 24 hr period[9]) by 0.01, as recommended in establishing water quality criteria[10]. An experimental-use sample of toxaphene (X-16189-49), furnished by Hercules Inc., was used throughout the study. Flow-splitting chambers designed by Benoit and Puglisi[11] were used to thoroughly mix and divide each toxaphene concentration for delivery to the replicated exposure tanks, and toxaphene concentrations in the exposure water were measured every two weeks. Acetone was used as the carrier solvent for toxaphene. The acetone concentrations in the control and highest toxaphene concentration tested were 0.23 ml/l in the fathead minnow study and 0.11 ml/l in the channel catfish study.

Water temperature in the tanks was controlled by heat exchangers and mixing valves before the water entered the systems. Artificial daylight was provided by the method of Drummond and Dawson[12]. The water temperature regime and photoperiod were those recommended by EPA for fathead minnow

tests[13]. The temperature regime for channel catfish was developed on the basis of data on natural water temperatures for channel catfish spawning (Table 1), and the photoperiod was the United States average (Evansville, Indiana), as recommended by EPA[13, 14].

We also conducted acute toxicity tests of toxaphene on 30-day-old fathead minnows and 2.5-year-old channel catfish[15]. The lethal threshold concentration was determined by following the recommendations of Eaton[9]. A proportional diluter system delivering seven concentrations and a control and with a dilution factor of 0.75, was used for the fathead minnows, as described by Johnson and Julin[16]. The test was conducted at 25 C, 20 fish were exposed to each concentration, and mortalities were recorded daily. The channel catfish test was run in a proportional diluter system having five concentrations (dilution factor of 0.75 between concentrations) and a control, with five fish per duplicate tank, at 20 C. The exposure tanks and system were the same as those for brook trout[3].

The design of the chronic toxicity studies was a randomized block design[17]. Growth and biochemical data of both the adults and fry were analyzed by analysis of variance, and the effects of toxaphene on mortality and egg hatchability were determined by conducting an analysis of variance on the arcsin transformation for proportions[18] (angle = arcsin $\sqrt{\text{percentage}}$). A multiple means comparison test (least significant difference) was used to compare treatments. The LC50's for the acute toxicity test were calculated by the method of Litchfield and Wilcoxon[19].

Fathead Minnows

This portion of the study was conducted according to the recommended procedure for chronic tests of fathead minnows[13]. Measured toxaphene concentrations (standard error) were 0, 13 (2), 25 (2), 54 (4), 97 (8), and 173 (12) ng/l. Water temperature was maintained at 25 ($\pm$0.5) C throughout the study. The 12 glass tanks used for exposing the fish measured 30 x 91 cm, were 30 cm deep, and had a water depth of 22 cm. Each tank was divided into three compartments. One compartment was 30 cm wide x 61 cm long and the other two were each 15 cm wide x 30 cm long.

Forty 40-day-old fry (0.32 g, 30 mm) were randomly selected and distributed to each of the large compartments in each tank for biochemical and residue determinations, and 10 fry were distributed to each of the smaller compartments for growth measurements. These fish were weighed and measured (total length) at 0, 30, 98, and 295 days of exposure. After 98 days of exposure, 4 males and 10 females were randomly selected and placed in the large compartment of each tank with five spawning tiles. All other fish were removed. Fifty eggs from each spawn were used for hatchability tests, and 20 of the resulting fry were placed in a small compartment and weighed and measured after 30 days to determine growth effects.

Channel Catfish

The toxaphene exposure was initiated 12 February 1974 and the measured concentrations (standard error) were 0, 49 (6), 72 (9), 129 (17), 299 (22),

TABLE 1. PHOTOPERIOD AND TEMPERATURE REGIME FOR PARTIAL CHRONIC TOXICITY TEST OF CHANNEL CATFISH

Month and date	Daylight (time)	Temperature (°C)
February		
1	0600-1715	17
15	0600-1745	
March		
1	0600-1815	17
15	0600-1900	
April		
1	0600-1930	19
15	0600-2015	
May		
1	0600-2045	22
15	0600-2115	26
June		
1	0600-2130	26
15	0600-2145	
July		
1	0600-2145	26
15	0600-2130	
August		
1	0600-2100	26
15	0600-2030	
September		
1	0600-2000	26
15	0600-1930	

and 630 (58) ng/l. Twelve fish (1,233 g, 461 mm) were placed into each of the 12 stainless steel test tanks (91 x 183 cm and 61 cm deep); 4 males and 4 females were used for evaluation of growth and spawning and 4 fish for analysis of residues. The water depth of the tanks was 46 cm, giving a volume of 765 liters. Well water was delivered to each tank at a rate of 1.6 l/min. The fish used for growth and spawning were weighed and measured (fork length) at 0, 50, and 100 days of exposure. The males were also weighed after day 75, and two of the four were removed for the toxaphene elimination study. After 50 days of exposure, the tanks were divided into two sections (91 x 91 cm and 46 cm deep) and the males, because of their increasing pugnaciousness, were separated from the females. After the final growth determination (100 days), dividers were inserted into one-half of the tank, dividing it into two sections for spawning, each 46 x 91 cm and 46 cm deep. One male and one female were placed into each of these sections. Sexual maturity was determined by the physical appearance of the genitalia, flaccidness of the abdomen of females, and darkening and swelling of the heads of males[20, 21]. Females judged ready to spawn were paired with a slightly larger ripe male. An 18.9-liter stainless steel milk can was then placed into each section with the paired fish, and half of each section was covered with a sheet of black plastic to reduce excitability. Cans were checked daily for eggs.

Females that had not spawned by the end of June were injected 3 to 4 times on alternate days with human chorionic gonadotropin at 300 IU/454 g[21]. Of the 15 fish injected, only 2 spawned, and the data were discarded because the hatchability of the eggs was very low. Egg masses were removed from the milk cans daily and numbers were determined volumetrically. Three 100-egg samples from each spawn were counted and placed into plastic containers (11 cm square and 16 cm deep; water depth, 11 cm) for hatchability determinations. The bottom was removed from each container and a rectangular slit (3.8 x 6.4 cm) was cut in one side just below the water surface. Stainless steel screen (7.9 meshes/cm) was used to cover the bottom and slit. An air stone was attached to the bottom screen and air was used to roll the eggs and circulate water (215 ml/min, or one exchange per 6.5 min) through the container. The fry hatched in 6 to 7 days, were counted, and returned to the hatching containers where they were kept for 5 to 6 days until swim-up. Fifty fry from each spawn were then weighed and placed in glass growth chambers (15 x 38 cm and 14 cm deep; water depth, 10 cm) with a flow rate of 200 ml/min. The remaining fry were placed in one section of each large tank for biochemical and residue determinations. The number of fry in each growth chamber was reduced to 20 at 30 days and 10 at 60 days when growth determinations (weight and fork length) were made. Final measurements were taken after 90 days of exposure. Mortalities were recorded daily.

BIOCHEMISTRY

Fathead Minnows

Backbones (vertebrae) were dissected from 10 adults from each concentration after 98 and 295 days of exposure and from 5 fry after 30 days of exposure. Collagen, calcium, and phosphorus were determined for the backbone of each adult and hydroxyproline was determined on each isolated collagen

fraction, as described by Mayer et al.[3] and Mehrle and Mayer[22]. Only the amount of hydroxyproline in the dried backbone was analyzed in fry. The backbone was dried at 110 C for 2 hr in a forced-air oven, split into two fractions, and weighed. Collagen was isolated from one fraction by the method of Flanagan and Nichols[23]. The isolated collagen was weighed and subjected to hydrolysis at 115 C in 5 ml of 6 N HCl for 16 hr. Hydroxyproline was determined in a 2-ml sample after Woessner[24]. The other bone fraction was subjected to hydrolysis at 115 C in 3 ml of 6 N HCl for 16 hr. Calcium was determined by atomic absorption spectrophotometry, and phosphorus was determined on the hydrolysate by the Fiske and Subbarow method[25]. The precision of each method varied less than 3%, and the recovery from spiked samples was 95-99%.

Channel Catfish

Backbone and blood samples were taken from female channel catfish within 24 hr after spawning. Collagen, hydroxyproline, calcium, and phosphorus of the backbones were analyzed as described for fathead minnows. Blood was sampled from the caudal artery and allowed to clot at room temperature; the serum was then decanted and frozen. Calcium and phosphorus were determined in the serum, as well as total protein[26] and vitamin C[27]. Six samples of three eggs or fry each were sampled from each concentration, and vitamin C analysis was performed on the eggs and on fry 15, 30, and 60 days old. Total protein[28] and hydroxyproline were determined in the eggs and in fry 15 days after hatch. Backbones were dissected from 10 90-day-old fry from each concentration, and collagen, calcium, and phosphorus determined in each bone sample and hydroxyproline was analyzed in each of the isolated collagen fractions. In addition, eight 90-day-old fry from each concentration were x-rayed for backbone anomalies.

RESIDUE DYNAMICS

General

Methodology for determination of toxaphene residues in water and fish was described by Stalling and Huckins[29]. The fish were ground and the tissues extracted by the procedures of Benville and Tindle[30] and Hesselberg and Johnson[31]. Initial sample cleanup was by automated gel permeation chromatography[32], followed by modified silicic acid chromatography[33]. Toxaphene residues were quantified by gas liquid chromatography with ^{63}Ni-electron capture detection. A 2.1-m long x 2-mm i.d. coiled glass column packed with 3% (w/w) OV-7 on chromosorb W-HP was used; nitrogen flow rate was 40 ml/min and the column temperature was 200 C. The percentage of extractable lipids was determined in each residue sample[34]. The minimum detection limit of toxaphene was 10 ng/l in water and 0.05 ug/g in fish tissue, but values below 0.1 ug/g in tissue were difficult to quantitate. Recovery of toxaphene from spiked tissue samples was 97-100%, and from water spiked at 25, 50, and 100 ng/l was 44-57%, 76-85%, and 97-104%, respectively. All residue values were adjusted for recovery.

Fathead Minnows

A composite sample of 10 fish was collected from each concentration at 30, 98, and 295 days of exposure. The fish remaining after sampling at day 295 were placed in fresh flowing water and a combined sample of 4 fish originating from each concentration was analyzed at 14, 28, and 56 days to determine the elimination rate of toxaphene. All excess eggs from each spawn within a concentration were pooled for analysis and 2 composite samples (40 to 60 fry each) of the resulting fry were collected from each concentration at the end of the 30-day growth period.

Channel Catfish

Two adults were sampled from each concentration after 30, 50, 75, and 100 days of exposure. On day 75, the excess males were placed in fresh flowing water for 33 days; 2 fish from each previous exposure were then analyzed for toxaphene elimination. After spawning (137 days), 2 males were removed from both the 72-and 299-ng/l exposures and toxaphene residues were determined in both the fillets and the remaining carcass(offal). Samples of eggs from each spawn were analyzed individually and two composite samples of fry from each concentration were analyzed after 15, 30, 60, and 90 days of exposure.

SECTION 5

RESULTS AND DISCUSSION

GROWTH, REPRODUCTION, AND MORTALITY

Parent fathead minnows held in toxaphene concentrations of 97 and 173 ng/l were significantly smaller than the controls ($P<0.05$) after 30 and 98 days (before spawning) but not at 295 days, toward the end of the spawning period (Table 2). No effects were observed on the spawns per female, eggs per female, eggs per spawn, female survival, or percent hatch (Table 3). Fry survival was significantly decreased only in the 97 ng/l toxaphene concentration; concentrations (ng/l) and mortalities (percent) were as follows: 0, 14; 13, 12; 25, 24; 54, 18; 97, 41; and 173, 21. Growth was significantly reduced ($P<0.05$) in fry from parents that had been exposed to 54, 97, and 173 ng/l (Table 2).

Growth of adult channel catfish was not affected by toxaphene; however, the fish had an average weight loss of 5.7% between the initiation of the study and the start of spawning. The fish fed poorly during the first part of the experiment, probably because the water temperature was low; as the temperature increased, the fish came into spawning condition and did not feed. The only aspect of spawning activity affected by toxaphene was the number of days between the pairing of adults and the start of spawning, which was significantly increased ($P<0.05$) in the highest concentration (Table 4). Egg hatch tended to decrease in the 129 ng/l and higher concentrations, but the decrease was not statistically significant. However, the proteinaceous matrix surrounding the eggs was greatly reduced in the highest toxaphene exposure (Fig. 2). The concentration of protein in matrix of the spawns of control fish and fish exposed to 630 ng/l did not differ--only the total amount of the matrix was reduced in the test fish.

The weight of channel catfish fry was significantly reduced ($P<0.05$) in the 299 and 630 ng/l toxaphene concentrations after 30 days of exposure and the same effect continued through 90 days (Table 5). Fry length was significantly reduced ($P<0.05$) in the 49, 129, 299, and 630 ng/l toxaphene concentrations after 30 days, but only in the 299 and 630 ng/l concentrations after exposure for 60 and 90 days. Cumulative mortality continued to increase throughout the 90-day exposure (Fig. 3), even though statistical significance was limited to the two highest concentrations. It appeared that the continuing mortality may have negated further decreases in growth by removing the less resistant fish. This hypothesis is supported by the effects of toxaphene on vitamin C (Table 10), which is essential in the detoxication of organic compounds in the liver by microsomal hydroxylative enzymes[35, 36],

TABLE 2. EFFECT OF TOXAPHENE ON GROWTH OF FATHEAD MINNOWS AS INDICATED BY WEIGHT AND LENGTH

Toxaphene concentration (ng/l)	Days of exposure						Size of fry of the 2nd generation at 30 days	
	30		98		295			
	Weight(g)	Length(mm)	Weight(g)	Length(mm)	Weight(g)	Length(mm)	Weight(g)	Length(mm)
0	0.51[a] (0.13)	36 (3)	1.02 (0.28)	44 (4)	2.62 (1.28)	58 (5)	0.172 (0.040)	25.3 (1.8)
13	0.52 (0.14)	36 (3)	1.12 (0.35)	45 (5)	2.35 (1.20)	56 (5)	0.165 (0.035)	24.9 (1.9)
25	0.47 (0.13)	35 (3)	0.95 (0.27)	42 (5)	2.21 (0.92)	55 (5)	0.174 (0.040)	25.1 (2.1)
54	0.49 (0.15)	35 (4)	1.01 (0.38)	43 (5)	2.51 (0.95)	58 (4)	0.161* (0.042)	24.7* (2.5)
97	0.44* (0.12)	35 (3)	0.86* (0.23)	41* (4)	2.30 (0.92)	57 (6)	0.152* (0.046)	24.1* (2.1)
173	0.40* (0.11)	34* (3)	0.79* (0.19)	40* (3)	2.08 (0.67)	55 (5)	0.155* (0.044)	24.4* (2.3)

[a]Mean (standard deviation).

*Significantly different from controls ($P<0.05$).

TABLE 3. SPAWNING ACTIVITY AND MORTALITY OF ADULT FEMALE FATHEAD MINNOWS EXPOSED TO TOXAPHENE

Toxaphene concentration (ng/l)	Spawns per female (n)	Eggs per female (n)	Eggs per spawn (n)	Female mortality[a] (n)	Egg hatch (%)
0	2.7	256	94	1	79
13	1.6	125	79	3	91
25	2.3	165	71	1	78
54	4.9	604	123	5	79
97	3.3	301	90	2	89
173	3.2	258	82	1	83

[a]20 females in each concentration.

TABLE 4. SPAWNING ACTIVITY AND MORTALITY OF ADULT FEMALE CHANNEL CATFISH EXPOSED TO TOXAPHENE

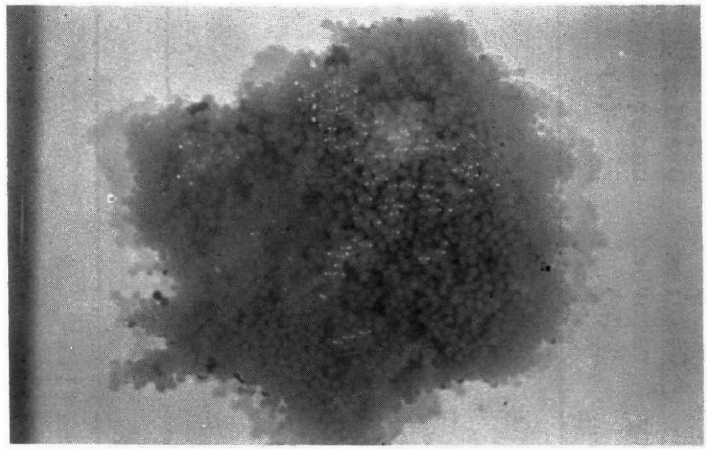
Toxaphene concentration (ng/l)	Spawns		Days to spawn after pairing	Female mortality (n)	Ovaries resorbed (n)	Ovaries underdeveloped (n)	Egg hatch (%)
	Potential number[a]	Actual number[b]					
0	8	6	12.4 (5.9)[c]	0	2	0	93
49	7	5	7.4 (3.9)	1	0	1	95
72	8	4	10.8 (5.0)	1	2	1	93
129	8	6	7.0 (2.0)	1	0	1	90
299	8	4	9.2 (3.2)	0	0	4	91
630	8	4	19.0 (4.5)*	2	1	1	75

[a]Number of females before spawning.

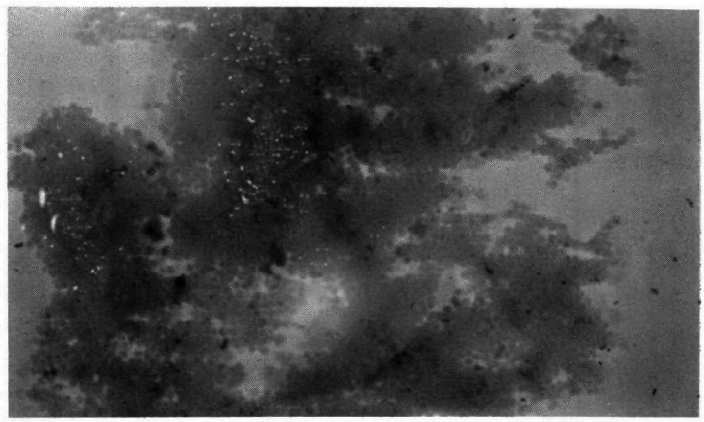
[b]One spawn was from a female injected with human chorionic gonadotropin in the control and 129 ng/l exposures.

[c]Mean (standard deviation).

*Significantly different from controls ($P<0.05$)



Control spawn



Spawn from 630 ng/l exposure

Figure 2. Effect of toxaphene on the proteinaceous matrix surrounding the eggs of channel catfish.

TABLE 5. EFFECT OF TOXAPHENE ON GROWTH OF CHANNEL CATFISH FRY AS INDICATED BY WEIGHT AND LENGTH

Toxaphene concentration (ng/l)	Days of exposure						
	5	30		60		90	
	Weight(g)	Weight(g)	Length(mm)	Weight(g)	Length(mm)	Weight(g)	Length(mm)
0	0.021[a] (0.004)	0.13 (0.05)	23 (3)	0.65 (0.22)	37 (4)	1.56 (0.67)	48 (7)
49	0.020 (0.003)	0.11 (0.04)	20* (3)	0.61 (0.17)	36 (4)	1.48 (0.41)	51 (5)
72	0.021 (0.003)	0.13 (0.04)	22 (2)	0.63 (0.20)	37 (4)	1.48 (0.42)	50 (5)
129	0.020 (0.002)	0.11 (0.03)	21* (3)	0.59 (0.18)	36 (4)	1.50 (0.44)	50 (4)
299	0.021 (0.003)	0.09* (0.03)	19* (2)	0.46* (0.22)	33* (5)	1.00* (0.21)	43* (3)
630	0.020 (0.004)	0.10* (0.02)	20* (2)	0.49* (0.09)	34* (6)	1.10* (0.35)	45* (4)

[a]Mean (standard deviation).

*Significantly different from controls ($P<0.05$).

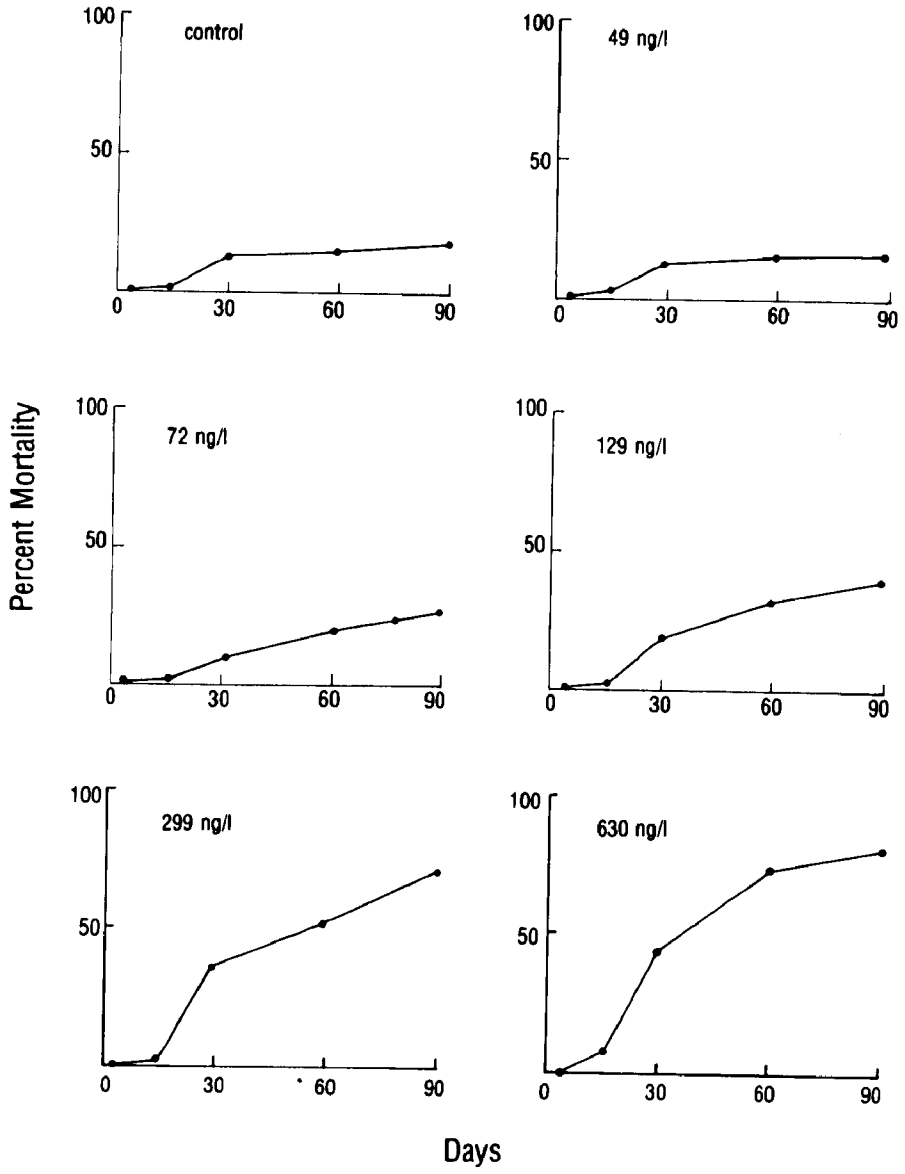


Figure 3. Cumulative mortality in channel catfish fry after 30, 60, and 90 days of exposure to toxaphene.

[37, 38]. The whole-body vitamin C concentrations in fry were significantly reduced ($P<0.05$) in the 72 ng/l and higher concentrations at 15 and 30 days of exposure, but the effect decreased at 60 days, indicating that the remaining fry were less susceptible to the effects of toxaphene.

Fishes were more sensitive to toxaphene than aquatic invertebrates. The acute toxicity of toxaphene to daphnids, scuds, and midges ranged from 10 to 180 ug/l[39], whereas the lethal threshold concentrations for brook trout[3], fathead minnows, and channel catfish were 4.1, 5.3, and 15.2 ug/l, respectively (Table 6). Daphnids were also more tolerant of toxaphene than fishes under field conditions[40].

BIOCHEMISTRY

Collagen was significantly reduced ($P<0.05$) in the backbones of fathead minnows exposed to 97 and 173 ng/l, and hydroxyproline in the isolated collagen was reduced in the 54, 97, and 173 ng/l toxaphene concentrations after 98 days of exposure (Table 7). However, no effects were found in the adult fathead minnows at the end of the spawning period. Calcium and phosphorus concentrations in the backbone were not affected by toxaphene as they were in a previous study[22], but the toxaphene exposures in that study were higher--55, 132, 288, and 621 ng/l (or 94, 205, 399, 727, and 1,420 ng/l, corrected for recovery). Due to the small size of the fry, only hydroxyproline was determined; it was significantly reduced ($P<0.05$) in fish exposed to 97 and 173 ng/l. Since we did not begin exposure of the parent fathead minnows to toxaphene until they were 30 days old and since the most important time of backbone development is in the early life stages, we placed fry after 30 days of exposure to the 0, 54, and 173 ng/l toxaphene concentrations in fresh water to determine if the reduction in hydroxyproline was of permanent significance in the development of broken backs. After 66 days in fresh water, the percentage of observable spinal curvatures was 14, 10, and 100% in the 0, 54, and 173 ng/l exposures, respectively.

At the concentrations tested, toxaphene had no effect on backbone composition or serum calcium, phosphorus, or protein in adult channel catfish. However, concentrations of 72, 129, 299, and 630 ng/l significantly reduced ($P<0.05$) hydroxyproline in the eggs and 15-day-old fry (Table 8). Collagen and hydroxyproline were also significantly decreased and calcium was increased in these concentrations in the 90-day-old fry (Table 9). Backbone phosphorus was reduced in the 129, 299, and 630 ng/l exposures. The incidence of backbone anomalies was not directly related to the exposure concentrations. Of the 8 fish x-rayed in each concentration, 0, 1, 5, 7, 5, and 5 fish showed deformed backbones in concentrations of 0, 49, 72, 129, 299, and 630 ng/l, respectively. In many fish, portions of vertebrae were missing in the backbone, especially at the anterior and posterior regions (Fig. 4). The effects on bone composition in fathead minnows and channel catfish were similar to those reported for toxaphene in brook trout[3, 41] and in a previous study with fathead minnows[22] where, in general, collagen in the backbone decreased.

TABLE 6. ACUTE TOXICITY OF TOXAPHENE TO 30-DAY-OLD FATHEAD MINNOWS AND 2.5-YEAR-OLD CHANNEL CATFISH

Days	Fathead minnows[a]		Channel catfish[b]	
	LC50 (ug/l)	95% confidence limits	LC50 (ug/l)	95% confidence limits
1	17.4	14.8-20.4	34.0	27.3-42.3
2	9.2	7.7-11.0	22.8	19.2-27.0
3	8.6	7.1-10.4	17.4	14.4-21.0
4	7.2	6.1-8.5	16.5	14.7-18.6
5	6.4	5.3-7.7	15.2[c]	13.8-16.7
6	5.6	4.7-6.7	15.2	13.8-16.7
7	5.3[c]	4.4-6.3	15.2	13.8-16.7
8	4.9	3.9-6.1	15.0	13.6-16.5
9	4.8	3.8-6.0	15.0	13.6-16.5
10	4.8	3.8-6.0		

[a]Mean weight, 0.32 g; length, 30 mm.

[b]Mean weight, 767 g; length, 394 mm.

[c]Lethal threshold concentration according to requirements described by Eaton[9].

TABLE 7. CONSTITUENTS (mg/g of dried bone) OF BACKBONE IN FATHEAD MINNOWS EXPOSED TO TOXAPHENE

Toxaphene concentration (ng/l)	Constituents and days of exposure								
	Collagen		Hydroxyproline[a]			Calcium		Phosphorus	
	98	295	98	295	30(fry)	98	295	98	295
0	190(32)[b]	290(19)	30(3)	27(4)	6.0(0.6)	76(8)	120(34)	41(4)	57(6)
13	220(51)	310(39)	29(4)	27(4)	5.4(2.2)	83(16)	110(11)	41(4)	59(7)
25	200(42)	310(31)	29(2)	27(2)	6.2(1.9)	76(11)	140(5)	40(3)	61(6)
54	180(31)	270(21)	24(6)*	28(2)	4.3(0.8)	85(12)	130(12)	41(4)	68(5)
97	140(26)*	270(22)	24(4)*	26(3)	3.9(1.0)*	81(8)	110(8)	39(6)	59(3)
173	150(27)*	260(26)	25(3)*	26(5)	2.2(1.1)*	79(10)	130(19)	39(6)	75(10)

[a]Hydroxyproline is expressed as mg/g of dried collagen in the adult fish.

[b]Mean(standard deviation).

*Significantly different from controls ($P<0.05$).

TABLE 8. HYDROXYPROLINE (mg/g protein) IN CHANNEL CATFISH EGGS AND FRY AS AFFECTED BY TOXAPHENE

Toxaphene concentration (ng/l)	Life stage	
	Eggs	Fry (15-day-old)
0	0.37(0.07)[a]	8.5(0.4)
49	0.35(0.05)	7.8(1.1)
72	0.29(0.08)*	2.3(1.3)*
129	0.30(0.03)*	1.2(0.1)*
299	0.23(0.05)*	2.2(0.2)*
630	0.27(0.08)*	6.5(0.8)*

[a]Mean(standard deviation).

*Significantly different from controls ($P<0.05$).

TABLE 9. CONSTITUENTS (mg/g of dried bone) OF BACKBONE IN 90-DAY-OLD CHANNEL CATFISH FRY EXPOSED TO TOXAPHENE

Toxaphene concentration (ng/l)	Constituents			
	Collagen	Hydroxyproline[a]	Calcium	Phosphorus
0	270 (16)[b]	57.6 (5.0)	65 (6)	64 (4)
49	260 (10)	53.4 (6.2)	81 (2)*	66 (5)
72	240 (16)*	47.3 (4.6)*	110 (2)*	63 (3)
129	240 (16)*	51.0 (5.2)*	95 (4)*	60 (4)*
299	240 (22)*	52.6 (6.8)*	85 (9)*	57 (3)*
630	230 (30)*	50.6 (4.6)*	81 (7)*	54 (2)*

[a]Hydroxyproline is expressed as mg/g of dried collagen.

[b]Mean(standard deviation).

*Significantly different from controls ($P<0.05$).

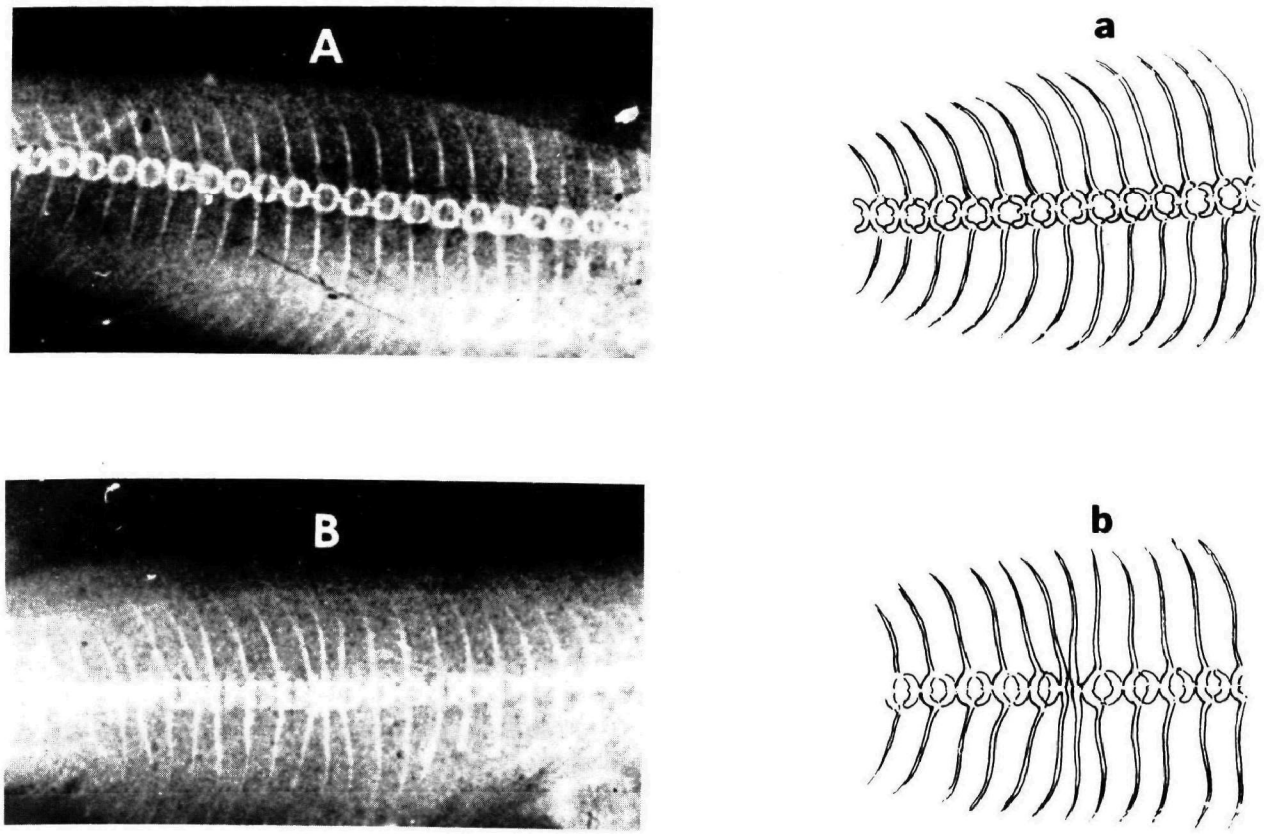


Figure 4. X-rays and schematics of backbones of 90-day-old channel catfish, normal (Aa) and exposed to 72 ng/1 of toxaphene (Bb).

It was suggested that a competition for vitamin C might exist between detoxication of toxaphene through hydroxylative enzymes and collagen formation through hydroxylation of proline to hydroxyproline[22]. Vitamin C is reported to be essential in the hydroxylation of drugs and other organic chemicals in the liver of mammals[35, 36, 37, 38] and in collagen formation via the hydroxylation of proline to hydroxyproline[42, 43, 44, 45]. Toxaphene significantly reduced whole-body vitamin C in channel catfish fry (Table 10). In another study with 10-month-old channel catfish[46, 47], vitamin C was significantly reduced ($P<0.05$) in bone and increased in liver by toxaphene. Therefore, the brittle backbone and backbone anomalies observed in studies with toxaphene appear to be caused by the reduction of vitamin C in bone, which inhibits the formation of hydroxyproline from proline and reduces collagen formation. Other organic chemicals which are detoxified by hydroxylative enzymes requiring vitamin C in the liver would be expected to produce backbone effects similar to those produced by toxaphene.

RESIDUE DYNAMICS

After 98 days of exposure, toxaphene in water was accumulated by fathead minnows from 69,000 times (0.9 ug/g) in the low concentration to 55,000 times (9.6 ug/g) in the high concentration (Table 11). One reason why the effects of toxaphene on growth and bone composition decreased from day 98 (before spawning) to day 295 (after spawning) may have been due to the decrease in toxaphene residues in the fish even though the exposure concentrations remained constant. Many organochlorine residues, such as those of toxaphene, are associated with lipids in animals. The amount of lipid decreased in fathead minnows from day 98 to day 295 (Table 12), probably because of the extra energy required for gonad development and spawning. On the basis of concentration in lipids, toxaphene residues also decreased during spawning (Table 13), but not as much as that observed on a wet-weight basis. Toxaphene residues ranged from 0.1 to 1.0 ug/g in eggs and from 0.2 to 2.8 ug/g in fry. The percentage declines in whole-body residues in adult fathead minnows 56 days after transfer to uncontaminated water were 0, 0, 25, 25, and 36% in the 13, 25, 54, 97, and 173 ng/l exposures, respectively.

Adult channel catfish accumulated toxaphene from water after 100 days of exposure by factors of 17,000 to 26,000 (Table 14). The factor of accumulation of toxaphene by fry from water ranged from 27,000 to 50,000. Concentrations of toxaphene in eggs were 0.24 ug/g in the controls and 4.4 ug/g in the highest exposure concentration, and the residues in fry continuously exposed to toxaphene did not equal or exceed the concentrations in eggs until 30 days after hatch. The lipid content of fry was also lower than that of eggs until the fry were 30 to 60 days old (Table 15); however, residues in eggs and fry differed little on a lipid basis, except in the two highest toxaphene concentrations (Table 16).

Adult male catfish exposed to 72 and 299 ng/l toxaphene were analyzed for residue distribution in the fillet and offal after exposure for 137 days. The toxaphene concentrations in offal were 4.4 and 4.1 times those in the fillets, which were 0.55 and 2.4 ug/g in fish exposed to 72 and 299 ng/l, respectively. After the adult male catfish were transferred to fresh flowing water for 33 days, the average percent decline in whole body residues was only 18% (Table 14).

TABLE 10. VITAMIN C CONCENTRATION (ug/g) IN EGGS AND FRY OF CHANNEL CATFISH EXPOSED TO TOXAPHENE

Toxaphene concentration (ng/l)	Eggs	Fry (exposure days) 15	30	60
0	38 (13)[a]	47 (16)	79 (20)	60 (13)
49	25 (3)	47 (8)	68 (16)	61 (10)
72	28 (6)	32 (8)*	55 (9)*	47 (18)
129	25 (17)	36 (4)*	60 (8)*	37 (5)*
299	38 (8)	27 (2)*	48 (16)*	44 (11)*
630	41 (13)	32 (9)*	45 (5)*	47 (3)

[a]Mean (standard deviation).

*Significantly different from controls ($P<0.05$).

TABLE 11. WHOLE-BODY RESIDUES (ug/g) OF TOXAPHENE IN FATHEAD MINNOW ADULTS AND THEIR EGGS AND FRY DURING EXPOSURE TO TOXAPHENE[a]

Toxaphene concentration (ng/l)	Adult treatment						Eggs	Fry (30-day exposure)
	Exposure (days)			Post exposure (days after start of exposure)				
	30	98	295[b]	309	323	351		
13	0.2	0.9	0.1	0.1	0.1	0.1	0.1	0.2
25	0.4	1.3	0.2	0.2	0.1	0.2	0.1	0.4
54	1.0	2.7	0.4	0.2	0.4	0.3	0.2	1.0
97	3.3	3.3	0.8	0.8	0.4	0.6	0.5	1.5
173	6.0	9.6	1.4	1.2	1.1	0.9	1.0	2.8

[a]No toxaphene was detected in control samples.

[b]All exposures were terminated on day 295, and fish were transferred to uncontaminated water for determination of elimination rates.

TABLE 12. EXTRACTABLE LIPIDS (% of wet weight) IN FATHEAD MINNOW ADULTS AND THEIR EGGS AND FRY DURING EXPOSURE TO TOXAPHENE[a]

Toxaphene concentration (ng/l)	Adult treatment						Eggs	Fry (30-day exposure)
	Exposure (days)			Post exposure (days after start of exposure)				
	30	98	295[a]	309	323	351		
0	12.6	6.0	3.1	3.7	6.2	6.9	0.30	6.0
13	5.5	11.4	2.3	4.8	6.4	8.1	0.09	5.0
25	5.1	8.1	2.0	3.1	5.7	7.3	0.06	5.0
54	5.1	8.4	3.2	2.9	4.8	6.9	0.20	5.9
97	7.1	9.2	4.1	3.3	5.4	8.4	0.10	5.1
173	5.7	9.7	2.3	5.3	5.8	7.8	0.20	5.2

[a]All exposures were terminated on day 295, and fish were transferred to uncontaminated water for determination of elimination rates.

TABLE 13. WHOLE-BODY RESIDUES (ug/g) OF TOXAPHENE ON A LIPID BASIS IN FATHEAD MINNOW ADULTS AND THEIR EGGS AND FRY DURING EXPOSURE TO TOXAPHENE[a]

Toxaphene concentration (ng/l)	Adult treatment						Eggs	Fry (30-day exposure)
	Exposure (days)			Post exposure (days after start of exposure)				
	30	98	295[b]	309	323	351		
13	3.6	11	5.2	2.7	1.1	1.2	133	3.3
25	7.8	11	11	8.1	2.6	2.7	200	7.9
54	20	33	12	7.9	8.3	4.3	125	17
97	46	35	19	25	7.0	7.1	540	30
173	105	99	60	24	19	12	510	52

[a]No toxaphene was detected in control samples.

[b]All exposures were terminated on day 295 and fish were transferred to uncontaminated water for determination of elimination rates.

TABLE 14. WHOLE-BODY RESIDUES (ug/g) OF TOXAPHENE IN CHANNEL CATFISH ADULTS AND THEIR EGGS AND FRY DURING EXPOSURE TO TOXAPHENE

Toxaphene concentration (ng/l)	Adult exposure (days)				Eggs	Fry exposure (days)			
	30	50	75[a]	100		15	30	60	90
0	1.1	1.4	1.3	1.1	0.24	0.14	0.10	0.12	0.17
49	1.2	1.6	1.1	1.3	0.74	0.25	0.50	1.0	2.2
72	1.6	1.3	1.7	1.9	0.90	0.60	1.3	2.2	3.0
129	1.8	1.3	2.4	2.6	1.7	0.50	1.9	3.0	4.4
299	3.0	2.0	4.6	6.2	2.6	1.8	3.4	5.8	8.1
630	3.9	3.3	7.2	11	4.4	1.4	4.0	18	32

[a]Two males were removed from each exposure on day 75 and were transferred to uncontaminated water for determination of elimination rates. After 33 days, residues were 1.0, 1.6, 1.1, 3.8, and 6.9 ug/g in the 49 through 630 ng/l exposures.

TABLE 15. EXTRACTABLE LIPIDS (% of wet weight) IN CHANNEL CATFISH ADULTS AND THEIR EGGS AND FRY DURING EXPOSURE TO TOXAPHENE

Toxaphene concentration (ng/l)	Adult exposure (days)				Eggs	Fry exposure (days)			
	30	50	75	100		15	30	60	90
0	10.4	11.0	7.4	7.8	1.5	1.1	1.6	3.2	6.2
49	8.3	8.6	6.5	10.0	1.8	0.81	1.5	1.3	4.8
72	8.5	10.3	6.2	7.2	1.7	0.90	2.5	3.7	4.4
129	7.5	7.8	7.3	8.4	1.7	0.95	2.0	3.4	4.6
299	10.7	9.0	8.1	5.8	2.6	0.94	1.8	2.0	4.3
630	9.6	5.9	7.5	7.4	2.1	0.84	1.3	4.3	5.5

TABLE 16. WHOLE-BODY RESIDUES (ug/g) OF TOXAPHENE ON A LIPID BASIS IN CHANNEL CATFISH ADULTS AND THEIR EGGS AND FRY DURING EXPOSURE TO TOXAPHENE

Toxaphene concentration (ng/l)	Adult exposure (days)					Fry exposure (days)			
	30	50	75	100	Eggs	15	30	60	90
0	10	13	18	14	16	13	6.2	3.7	2.7
49	14	18	17	13	41	31	33	77	46
72	19	13	27	26	53	67	52	59	68
129	24	17	33	31	100	52	92	88	95
299	28	22	57	107	100	191	189	290	188
630	41	56	96	148	209	167	308	418	582

GENERAL

An application factor of 0.01 has been recommended in establishing water quality criteria for organochlorine insecticides[10]. The factor is derived by dividing the maximum acceptable toxicant concentration (MATC; the highest continuous toxicant concentration that has no adverse effect on growth, reproduction, and mortality) by the 96-hr LC50 value[48] or by the lethal threshold concentration[9]. Mount and Stephan[48] hypothesized that the application factor for a given toxicant experimentally determined for one fish species is applicable to other species of fish, and this hypothesis is further supported by our data (Table 17). In both invertebrates[39] and fishes, the calculated application factors bracketed or approached the recommended factor of 0.01.

The application factor concept is a potentially valuable aid in determining water quality criteria. It is almost impossible to experimentally determine the MATC of a contaminant for all aquatic organisms because of the long time required for conducting chronic toxicity tests. Within this hypothesis, however, the MATC for a species in question can be derived by conducting an acute toxicity test and multiplying the value by an application factor that is experimentally determined for one species only. The no effect level based on biochemical observations was consistent with the MATC derived on the basis of growth, reproduction, and mortality in brook trout and fathead minnows. The no effect level on a biochemical basis for channel catfish was between 49 and 72 ng/l but the MATC was 199 to 249 ng/l.

Mayer et al.[3] recommended that collagen and hydroxyproline in backbone be considered as early biochemical indicators of growth and developmental changes in fishes. The studies with brook trout[3], fathead minnows, and channel catfish show that observable effects on growth correlate well with the biochemical measurements. However, effects on collagen and hydroxyproline in channel catfish were significant at lower toxaphene concentrations than was growth, and the biochemical approach may give a more adequate assessment of the potential danger of a contaminant to fish in some situations.

TABLE 17. CONCENTRATIONS OF TOXAPHENE PRODUCING ACUTE AND CHRONIC TOXICITY TO AQUATIC ORGANISMS

Species	LTC[a] (ug/l)	Predicted MATC[b] (ng/l)	Observed MATC (ng/l)	Application factor[c]	Source (reference no.)
Daphnids, *Daphnia magna*	10[d]	100	>70<120	>0.007<0.012	39
Scud, *Gammarus pseudolimnaeus*	24[e]	240	>130<250	>0.005<0.010	39
Midge, *Chironomus plumosus*	180[e]	1,800	>1,000<3,200	>0.006<0.018	39
Brook trout	4.1	41	<39	<0.010	3
Fathead minnows	5.3	53	>25<54	>0.005<0.010	pp[f]
Channel catfish	15	150	>129<299	>0.009<0.020	pp

[a]Lethal threshold concentration[9].

[b]Predicted safe concentration, based on an arbitrary 0.01 application factor[10] times the LTC.

[c]Experimentally derived by application of MATC's and acute values.

[d]48-hr EC50.

[e]96-hr LC50

[f]Present paper.

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

Fathead minnows (Pimephales promelas) and channel catfish (Ictalurus punctatus) were continuously exposed to several toxaphene concentrations (13-630 ng/l) in flow-through diluter systems for 8 to 10 months. Growth and backbone quality of adult fathead minnows were decreased at 97 and 173 ng/l exposures, but adult channel catfish were not affected by toxaphene. Effects on reproduction were observed only in channel catfish in the 630 ng/l concentration: the period from pairing to spawning was increased and the amount of gelatinous matrix surrounding the eggs was reduced. Survival of fathead minnows was not affected by toxaphene, but the no-effect concentration for fry growth and bone quality was below 54 and 97 ng/l, respectively. Channel catfish fry survival and growth were reduced in the 299 and 630 ng/l exposures, and bone quality was altered in concentrations as low as 72 ng/l. The maximum toxaphene accumulation from water to fish was 69,000 times in fathead minnows and 50,000 times in channel catfish. Toxaphene was excreted very slowly in both species.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Growth Collagens Reproduction (biology) Mortality Pesticides Fishes	Catfish Fathead Minnow Toxaphene Chronic Effects Continuous Exposure Residue Dynamics Partial Life Cycle	6C 6F 6T

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