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ACUTE AND CHRONIC EFFECTS OF CHLORPYRIFOS

ON Tilapia zillii

presented by

Mohamed M. Shereif

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Doctor of Philosophy degree in Fisheries & Wildlife


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ACUTE AND CHRONIC EFFECTS OF CHLORPYRIFOS

ON Tilapia zillii

By

Mohamed M. Shereif

A DISSERTATION

**Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of**

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ABSTRACT**ACUTE AND CHRONIC EFFECTS OF CHLORPYRIFOS****ON Tilapia zillii****By****Mohamed M. Shereif**

The fish, Tilapia zillii (family: cichlidae) was exposed, in acute and chronic toxicity tests conducted under continuous flow conditions, to chlorpyrifos, a broad spectrum organophosphorus insecticide, used extensively in agriculture to control different kind of pests. In the chronic studies, the spawning and reproductive behavior, survival of early life stages, and growth of the fish were significantly impacted at a chlorpyrifos concentration which was two orders of magnitude smaller than the acute 96-h LC50. The specific growth rate of T.zillii was the most sensitive parameter measured for chlorpyrifos.

The maximum acceptable toxicant concentration (MATC) and "safe" level for chlorpyrifos were determined and both the application factor (AF) and chronic value were also estimated from the chronic studies.

In addition, the activity of brain acetylcholinesterase

(AChE) was significantly inhibited for adult and fingerling T.zillii exposed to chlorpyrifos for 90 and 35 days respectively compared to controls, and the enzyme inhibition was significantly higher in adult females than in males.

Residues of chlorpyrifos in both adult and fingerling T.zillii muscle tissues were measured, after their exposure for 90 and 35 days respectively, and the bioconcentration factors were estimated from chlorpyrifos concentrations in tissues and water. There was no significant difference between chlorpyrifos tissue residues in adult males and females. Also, there was no relationship between the feeding levels and chlorpyrifos residues in fingerling tissues.

DEDICATION

**To my wife, Nagwa, and our children Ghada, Ahmed,
and Salma**

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INTRODUCTION

Pesticide is a general term used to describe all the so-called economic poisons. Pesticides are primarily used to prevent, regulate, or eliminate pests. Some of the major kinds of pesticides are insecticides, herbicides, fungicides, miticides, nematocides, and rodenticides. Pesticide use worldwide each year is roughly a pound weight for every man, woman and child on earth, and the world pesticide market is growing in both volume and value; between 1972 and 1980 the market grew in real terms by an annual average growth rate of 5% (Bull, 1982). But the use of pesticides has created a paradoxical problem which man must solve if he is to retain the quality of life he desires (Lamb, 1972).

Pesticides have benefited mankind in many ways. In agriculture, pesticides have effectively reduced losses of crops from insects, weeds, and plant diseases, which have been estimated as high as 45% of the total food production (Pimentel and Levitan, 1986). Also, pesticides proved extremely effective in controlling human insect-borne diseases such as typhus, plague, and malaria. In the 1940's, DDT saved millions of lives in civilian and military populations from such epidemic diseases (Lamb, 1972).

On the other hand, the extensive use of pesticides has resulted in creating many unforeseen problems to mankind and

the environment.

Pimentel and Levitan (1986) noted that poisoning of humans is part of the high price paid for pesticide use and estimated that 45,000 total human poisonings occur annually worldwide, including about 3,000 cases admitted to hospitals and about 200 fatalities, with approximately 50 of the latter being attributed as accidental death.

In agriculture, many kinds of pests have developed resistance to pesticides. Dover and Croft (1986) reported that 428 species of arthropods have developed resistance to one or more insecticide or acaricide, over 150 plant resistant pathogen species are known, over 150 herbicide-resistant weed species have now been reported, and about 10 species of small mammals and plant-attacking nematodes are known to be resistant. In addition, as a result of intense pesticide application, pesticides caused the near elimination of many natural enemies of pests resulting in outbreaks of certain pests that were previously minor pests; about \$153 million is spent each year to control these newly created pests (Pimentel and Levitan, 1986).

The environmental contamination of air, land, and water by pesticides, and their deleterious effects on nontarget organisms, is probably the greatest public environmental concern problem since the 1960's. This pesticide contamination problem was caused primarily by some organochlorine insecticides (i.e., DDT), that persist so long

in nature (Nicholson, 1969). Rudd (1971) stated that the organochlorine insecticide DDT, and its metabolite DDE, "can now found in biological tissues from the open ocean to the polar ice caps, in airborne dusts over cities, and in plants, animals, and waters in remote forests and mountains". Other pesticides, such as the organic phosphorus insecticide group, which are relatively unstable in the environment, are rarely stored in animal tissues, and they are relatively less dangerous for non target organisms (Holden, 1964).

Nicholson (1967) suggested that the aquatic environment is the ultimate sink for all man-made pollutants, many of which are remarkably lethal to aquatic organisms. In addition, the adverse impact of pollutants, including all pesticide groups, is much greater on the aquatic organisms than on the terrestrial organisms (Murty, 1986). Fish and other aquatic forms of life in standing water are unable to escape from an insecticide once it has been released in water, and have to submit to its physiological adverse effects until it has been removed by adsorption on sediments, hydrolysis, or some other mechanisms (Brown, 1978).

It has been found necessary to develop some techniques and methods to detect and evaluate the hazard and degree of toxicity of various toxic chemicals and pesticides on fish and other aquatic organisms (Muirhead-Thomson, 1971).

Toxicity tests can be defined as those tests used to

evaluate the concentration of the chemical and duration of exposure time required to produce an adverse effect, and to measure the degree of response produced by a specific level of test chemical concentration (Rand and Petrocelli, 1986). Information generated from toxicity tests can be very useful in the management of environmental contamination by pollutants, especially in the prediction of environmental effects of a toxicant and comparison of toxicants or test organisms (Buikema et. al., 1982).

Most of the present modern aquatic toxicity test protocols are the result of different attempts at the standardization of the test methodology (Murty, 1986), but the earliest efforts by Doudoroff et. al., (1951), became the basis of all other methods. Other contributions to the standardization of toxicity test methodology and test conditions have been established by the American Public Health Association, American Water Works Association, and Water Pollution Control Federation "standard methods for the examination of water and wastewater", (1985); American Society for Testing and Materials (ASTM), (1980); and Organization for Economic Cooperation and Development (OECD), (1981).

Toxicity tests are divided into acute and chronic techniques primarily on the basis of the duration of the test. The acute toxicity tests are conducted over a period of 24 to 96 hours. These tests are commonly used to evaluate the toxicity of pollutants and provide some

information about their lethality. Acute tests are usually conducted to determine the toxicant concentration that is capable of affecting 50% of the exposed test organisms over the duration of the test. This concentration is known as the toxicant LC50 value. Stephan (1977) reviewed the different methods for calculating the median lethal concentration (LC50) and 95 percent confidence limits from concentration-mortality data produced by an acute toxicity test.

Acute toxicity tests may be designed and conducted in static, static renewal, recirculation, and continuous flow-through systems. The static and flow-through techniques are the most widely used in acute toxicity tests (Parrish, 1986). Macek et. al., (1978) stated that "static acute tests provide practical means for (1) deriving estimates of the upper limit of concentrations that produce toxic effects, (2) evaluating the relative toxicity of large numbers of test materials, (3) evaluating the effects of water quality, (4) evaluating the relative sensitivity of different aquatic organisms to test materials, and (5) developing an understanding of the concentration-response relationship and of the significance of duration of exposure to the test material". For the flow-through acute tests, Parrish (1986) stated that "these tests enhance the ability to maintain satisfactory test conditions and are not limited in duration; thus they allow a more complete assessment of the relationship between exposure duration and effect".

However, fish and other aquatic organisms are commonly subjected to long term stress arising from exposure to sublethal concentrations of toxicants which could be more deleterious to spawning or reproductive behavior, survival of early life stages, and growth of the fish (Murty, 1986). Therefore, chronic toxicity tests are now commonly conducted to provide a more sensitive measure of chemical toxicity than is allowed by acute toxicity tests (Petrocelli, 1986). Mount and Stephan (1967) stated that " Pollution biologists must have quantitative data to prove that the observed change resulting from exposure is ecologically detrimental to fish or the harvestable crop. An exposure causing death is obviously significant, but even the best fish physiologist would have difficulty establishing that a 10 percent reduction in hematocrit would result in an undesirable effect on a population".

Chronic toxicity test methodology is quite variable and is specific to the life history of the organism used in the test (i.e., eggs, larvae, fingerlings, or adults). A chronic toxicity test could be based on either total life cycle or partial life cycle tests over time periods of weeks to years, and are usually conducted in flow through systems to evaluate effects on such things as spawning, survival of the young, and growth of the test organism.

Chronic toxicity tests are of primary value in estimating the "safe" levels of toxicants, also known as the "no effect" concentration or the Maximum Acceptable Toxicant

Concentration, MATC (Mount and Stephan, 1967; Eaton, 1973). The MATC is empirically estimated, and is the highest concentration that has no observed effect on the exposed organism (NOEC) in terms of spawning, survival, or growth, or it is interpolated as the geometric mean (chronic value) of the lowest concentration that has an observed effect (LOEC) and the no observed effect concentration (NOEC) (Buikema et. al., 1982). In addition, when the maximum acceptable toxicant concentration (MATC) is divided by the concentration causing 50% mortality (LC50), the result is the application factor (AF) (Mount and Stephan, 1967).

Data produced from chronic toxicity tests are of great ecological importance where reductions in survival, growth, or reproductive success of fish or fish food organisms can lead to reduced fish recruitment and thus declining fish populations (Buikema et. al., 1982).

Fish play as important a role in aquatic toxicity testing and hazard evaluation as do the white rat and guinea pig in mammalian toxicology (Anon, 1978). Also, fish are presumed to be the best understood organism in the aquatic environment and are perceived as the most valuable output from aquatic systems by the majority of the public (Buikema et. al., 1982).

Of the great many different acute and chronic toxicity studies of pesticides with fish, very few studies been conducted with the fish tilapia (family: cichlidae), especially T.zillii, which is widely distributed in Africa

and Palestine, in South and Central America, in southern India and in Sri Lanka (Lagler et. al., 1977). Tilapia have become increasingly important in fish culture, especially in warm climates, have become a major source of protein in many of the developing countries (Pullin and Lowe-McConnel, 1982), and they are appreciated by both fish growers and consumers in many countries where they can produce high yields on relatively low inputs (Hepher and Purginin, 1982). In addition, T.zillii are substratum spawners, very prolific and can spawn many times during a year (El-Zarka, 1956; El-Bolock and Koura, 1960).

In the present study, the fish T.zillii was exposed, in laboratory acute and chronic toxicity tests, to the organophosphorus insecticide chlorpyrifos (Dursban), a broad-spectrum insecticide used extensively in agriculture to control a wide range of insects on crops, vegetables, and fruits, and commonly used also to control mosquitoes, fire ants, turf and ornamental plant insects, termites and cockroaches. Chlorpyrifos, which has an anticholinesterase mode of action, has been reported to be one of the most acute toxic organophosphorus insecticides to fish and other aquatic organisms (Marshall and Roberts, 1978). The 96-h LC50 was reported for some fish species to be as small as 1.3 ug/l for california grunion, Leuresthes tenuis (Borthwick et. al., 1985), and 2.4 ug/l for bluegills, Lepomis macrochirus (Johnson and Finely, 1980).

There was no published chlorpyrifos chronic test data

on nontarget aquatic organisms until 1982, when Jarvinen and Tanner conducted a 32-day embryo-larval study with fathead minnows, Pimephales promelas, and both technical grade and encapsulated formulation of chlorpyrifos, and reported some significant effects on growth of the fish at a concentration as low as 3.00 ug/l. In the same study, the chronic values for the chlorpyrifos technical grade and encapsulated formulation were estimated as 2.26 ug/l and 3.25 ug/l respectively.

The widespread dependence on both T.zillii for human food and on chlorpyrifos to control pests throughout the world suggests a potential for several deleterious interactions between these two dependences. As such, the the objectives of the present study were to:

- I. Determine the acute 96-h LC50 value of chlorpyrifos for T.zillii.
- II. Conduct different chronic toxicity tests using the continuous flow-through system to evaluate different chronic effects of chlorpyrifos on spawning and reproductive behavior, survival of early life stages, and growth of T.zillii.
- III. Use the chronic data to determine the "Safe" level of using chlorpyrifos, the chronic value, and the application factor (AF) for chlorpyrifos on T.zillii.
- IV. Evaluate the biochemical effect of chlorpyrifos

through the analysis of acetylcholineesterase (AchE) in brains of both adult and fingerling T.zillii.

- V. Analyze chlorpyrifos residues in the muscle tissues of both adults and fingerling T.zillii and estimate the bioconcentration factor (BCF) for chlorpyrifos.
- VI. Provide baseline data for application of chlorpyrifos in agriculture to protect aquatic forms of life and aquatic ecosystems.

MATERIALS AND METHODS

All experiments in this study were carried out at the Department of Fisheries and Wildlife and Pesticide Research Center, Michigan State University.

The T.zillii used with these experiments were obtained from two separate sources. T.zillii fingerlings used in the acute toxicity test were obtained from Santee Cooper Hatchery (George Town, South Carolina). The fish used in all other experiments were derived from adult fish provided generously by Fish Breeders Of Idaho (Buhl, Idaho). Some of the adults supplied were used in the chronic spawning and reproduction experiments, while others were saved and used as brood stock to supply both the larval fish used in the chronic early life stage experiments (ELS) and the fingerlings used in the growth experiments.

When the fish arrived from these two sources, they were acclimated to the laboratory conditions for two weeks in recirculating-water, living-stream tanks (Frigidunits Inc., Toledo, Ohio). These tanks were supplied with charcoal-filtered water and continuous aeration and water temperature was controlled to 27 ± 1 C by a 30 amp thermoregulator relay (H-B Instrument Co., Philadelphia, PA) equipped with a thermoregulator and water heater. A constant 16 h: 8 h (light:dark) photoperiod was maintained over fish tanks with fluorescent light fixtures and a timer. During the

acclimation period, fish were fed Purina Trout Chow, which was the same food used in all experiments. The fish were also acclimated for another week to the glass tanks used in all experiments at a constant temperature of $27 \pm 1^{\circ}\text{C}$ and a photoperiod of 16 h light: 8 h dark.

The organophosphate insecticide, chlorpyrifos (o,o-diethyl-o-3,5,6-trichloro-2-pyridyl) phosphorothioate used in these experiments was supplied by the Dow Chemical Company, Midland, Michigan as a technical grade material with a 97% purity. Some of the physical and chemical properties of chlorpyrifos are summarized in Table 1.

I. Acute Toxicity Experiment

The acute toxicity of chlorpyrifos to T.zillii was determined with the 96 h static toxicity test outlined by the United States Environmental Protection Agency (1982). Duplicate 20 gallon aquaria filled with 50 liters of charcoal-filtered tap water were used to expose the fingerlings of T.zillii to four concentrations of chlorpyrifos, 56.0, 100.0, 180.0, and 320.0 ug/l. These concentrations of chlorpyrifos were obtained by dissolving carefully measured volumes of the technical grade in four ml acetonitrile used as a solvent. An equal volume of acetonitrile containing the appropriate amount of the technical grade was added to each 50 liter aquaria to obtain the desired concentrations. The same amount of the solvent, acetonitrile, was added to solvent control aquaria. In

Table 1. Some physical and chemical properties of chlorpyrifos (from Brust, 1966; Marshall and Roberts, 1978).

Molecular weight	350.57
State at 25 C	white granular crystalline solid
Melting point (C)	41.5-43.5
Heat of sublimation	26,800 calories/mole
Water solubility at 23-25 C (mg/l)	0.4-2.0
N-octanol/water partition coefficient	66,000
Soil organic carbon/ water partition coefficient	13,600

addition, separate water controls were used, and all tanks were maintained at $27 \pm 1^{\circ}\text{C}$ in a controlled temperature water bath.

The fish were not fed during the acute toxicity experiment and all symptoms of toxicity were observed and recorded at four hour intervals for the first day and at 24 hour intervals for each day thereafter. Fish were considered dead when there was no movement of the operculum, and dead fish were removed. The mortality data were recorded at 24 hour intervals.

II. Chronic Toxicity Experiments

Experiments were conducted to evaluate the chronic sublethal effects of chlorpyrifos on spawning and reproductive behavior, early life stages, and growth of the fish *T. zillii* under continuous flow-through conditions. A proportional diluter (Mount and Brungs, 1967; Lemke et. al., 1978) of the solenoid valve type (Ace Glass Inc., Vinland, N.J.) was constructed and used for all chronic experiments. This diluter allowed maintenance of constant concentrations of chlorpyrifos in the exposure aquaria.

Dissolved oxygen, pH, and total alkalinity and hardness of water in all exposure tanks were monitored weekly (American Public Health Association, American Water Works Association, and Water Pollution Federation, 1985), while temperature was monitored daily. The range of values recorded for these parameters is given in Table 2.

Table 2. Some chemical characteristics of the water used in the flow-through diluter system during the chronic toxicity experiments.

Dissolved oxygen (mg/l)	7.1-8.2
Total alkalinity (mg/l as CaCo3)	321-330
Total hardness (mg/l as CaCo3)	360-365
pH	7.8-8.5
Temperature (C)	27 ± 1

1. The diluter system

The diluter consists of series of cylindrical glass vessels in three layers, (Figure 1). A series of diluent water vessels were located in the upper layer, the toxicant vessels were in the middle, and the mixing and splitting vessels were in the bottom layer which was located between the diluter and the exposure tanks. All vessels were graduated and equipped with standpipes for instant determination and adjustment of outflows. In addition, there were two more vessels, the pre-toxicant vessel which received the toxicant directly from the carboy stock solution, and the pre-toxicant mixing vessel which received the first toxicant dilution. Stainless steel tubes were used to transfer the dilution water or toxicant between vessels.

The diluter was equipped with an automatic control panel which controlled the normally closed (NC) and normally open (NO) solenoids, the sensory electrodes, and filling and dispensing of diluent water and toxicant. Three sequences of cycles were maintained by the control panel, the filling cycle, the leveling cycle, and the dumping cycle. In the filling cycle, the normally open (NO) solenoids opened, the normally closed (NC) solenoids closed, and the diluent water and toxicant vessels were filled. When the sensory electrodes contacted the two sets of liquid levels, the leveling cycle started. In this cycle, the NO solenoids closed, and all liquid levels stabilized within 50 seconds.

**Figure 1. Diagram of the proportional diluter system
used in the chronic toxicity experiments.**

A: dilution water vessels

B: chemical vessels

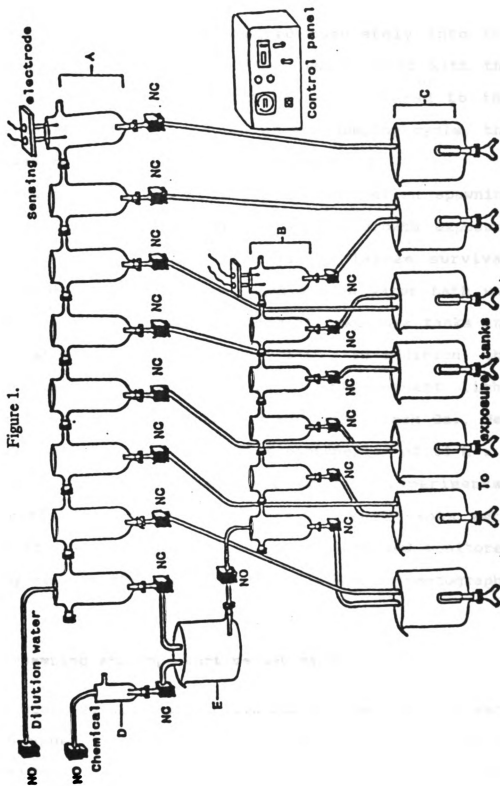
C: mixing and splitting vessels

D: pre-chemical vessel

E: pre-toxicant mixing vessel

NC: normally closed solenoid

NO: normally open solenoid



The dumping cycle began after the leveling cycle was completed. The NC solenoids then opened and both diluent water and toxicant vessels drained completely into the mixing and splitting chambers from which water with the appropriate toxicant concentration is delivered to the exposure tanks. Upon completion of the dumping cycle, the process was reinitiated with the filling cycle.

Complete turnover was 11 h for the 70 gallon spawning exposure tanks, five h for the 20 gallon growth exposure tanks, and one h for the five gallon larvae survival exposure tanks. A fiberglass lined wooden water bath was built along with the diluter to hold all exposure tanks and maintain water temperature at 27 ± 1 C. In addition, the exposure tanks were supplied with fluorescent light fixtures and an automatic timer, Model 1191 (Tork Co., New York, N.Y.), to maintain a constant photoperiod of 16 h : 8 h (light : dark) throughout all chronic experiments. Chlorpyrifos concentrations in the exposure tanks were measured at the beginning of each experiment and monitored weekly by reverse-phase High Pressure Liquid Chromatography (HPLC).

2. Spawning and reproductive behavior

The spawning and reproductive behavior of T.zillii were observed under two sublethal measured concentrations of chlorpyrifos, 1.7 and 3.6 ug/l using the proportional diluter system described above. Healthy mature males and

ripe females were transferred from the adult brood stock into duplicates of 70 gallon exposure tanks (approximately 120 liters). Sexing of fish was based on the structure and form of the genital papillae (Bardach et. al. , 1972). The choice of the mature and ripe fish was determined by pressing the belly of fish. If the fish were ripe, olive green eggs were stripped out from females and white milt was emitted in the case of males.

Only one pair consisting of one mature male and one ripe female was placed in each exposure tank. The bottom of the tank was covered with sand to enhance nest building by fish. Fish were fed daily to satiation with Purina Trout Chow. Tanks were covered by screens to avoid fish jumping from the tanks, and blinds were set around all tanks to prevent disturbance of the fish during spawning. In addition, the glass side walls of the tanks were covered with dark plastic screens to minimize any possible disturbance between spawners in the adjacent tanks. The fish spawned initially after 6-7 days in the tanks and spawned a second time 30-35 days later.

During the interspawn period (30-35 days), males were separated from females by nylon screen dividers to prevent injury to females by males during this period. The young fish were removed after the two week parental care period. All tanks were cleaned thoroughly between spawns.

3. Early life stages

In this experiment, newly hatched larvae of T.zillii (< 24 h) were collected from brood tanks and exposed to two sublethal measured concentrations of chlorpyrifos (1.7 and 3.6 ug/l) for 35 days. Fifty larvae were put in each of the 5 gallon tanks (filled to contain 8.5 liters) operated at a water overturn time of one h. The larvae exposure tanks were suspended inside the 70 gallon spawning tanks, two in each one. Because of the high sensitivity of these young fish, the larvae tanks were carefully cleaned at frequent intervals. After the larvae exhausted their yolk sacs, they were fed very fine powdered Purina Trout Chow to satiation, and any food left was siphoned from the tanks. Dead larvae were removed and recorded daily. At the end of the 35 day exposure time, the surviving larvae were collected and counted to calculate the percentage survival. The guidelines followed in this test were those outlined by United States Environmental Protection Agency (1982) with a temperature of $27 \pm 1^{\circ}\text{C}$ and the tank arrangement described above.

4. Growth

Fingerling T.zillii were fed six different rations of Purina Trout Chow, 0%, 1%, 2%, 3%, 4%, and 5% per day (dry weight feed/wet weight fish/day), and exposed to two sublethal measured concentrations of 1.7 and 3.6 ug/l of

chlorpyrifos for five weeks at a constant water temperature of $27 \pm 1^{\circ}\text{C}$. Five fingerlings (average weight: 6.5 g ; average length: 3.5 cm) were placed in each of the 20 gallon aquaria (filled up to 50 liters), and the different food levels were calculated based on the total wet weight of the fish in each tank.

Another set of control tanks was maintained with no chlorpyrifos addition in which the fish received the same food levels. Each tank was equipped with a 150 watt heater, a power filter, a stone aerator, and received a light intensity of 16 h L : 8 h D. Fish in each tank were fed twice a day (900 h and 1600 h), and were weighed weekly. Weights of fish and food were measured using an Ohaus balance, Model B-1500-D (Ohaus Scale Co., Floram Park, N.J.). Fecal matter and remnants of food were siphoned daily before the first feeding time (900 h).

III. Brain Acetylcholinesterase (AChE) Activity

At the end of the chronic spawning and growth studies, brains of fish (males, females and fingerlings) were removed from the exposure tanks, blotted free of blood, wrapped in aluminum foil, and stored in liquid nitrogen.

These brains then were homogenized in chilled 0.1 M phosphate buffer using a Wheaton electric homogenizer with a teflon pestle. The activity of AChE in the crude homogenate was estimated with the method described by Ellman et. al., (1961). The reagent, dithiobisnitrobenzoic

acid (DTNB) was added to a mixture of the brain homogenate and 0.1 M sodium phosphate buffer. The absorbance of the above reaction mixture was measured using a spectrophotometer at a wave length of 412 nm. After a steady reading was obtained, the substrate acetylthiocholine iodide was added and the change in absorbance was recorded using the same wave length.

The activity of acetylcholinesterase was calculated using the extinction coefficient of the yellow anion as described by Ellman et. al., (1961) and expressed as umole of acetylthiocholine hydrolyzed/ mg protein/ min. Protein was estimated by the method of Lowery et. al., (1951) using bovine serum albumin as a standard. The percentage AchE inhibition was calculated by comparison with the mean normal activity of control fish.

IV. Chlorpyrifos Residue Analysis

Chlorpyrifos residue concentrations in water were measured each week in each exposure tank during the chronic studies. Chlorpyrifos residue concentrations in the fish were measured at the end of the chronic spawning and growth studies.

1. Residues in water

Fifty-ml water samples were taken from each exposure tank and extracted three times with 25-ml portions of methylene chloride in a 25-ml separatory funnel. The

mixture then was shaken vigorously for one minute, and the two layers were completely separated. The methylene chloride extracts (the lower portion) were collected and combined in a round bottom flask. The solvent phase was then poured through a sodium sulfate anhydrous column. The extract was then concentrated by rotary evaporation and nitrogen evaporation at 40 C. The dried extract was dissolved with 2.0 ml of acetonitrile. Fifty microliters of this dissolved extract were injected into a reverse phase HPLC equipped with a UV detector adjusted to 290 nm. The injections were made into a 5 cm., C-18, 2 μ m guard column connected directly with an ULTREX 5 C-18 column, 250 x 4.6 mm., at ambient temperature.

An isocratic elution was used with 82.0/ 17.5/ 0.05 ml (v/ v/ v) acetonitrile/ water/ phosphoric acid. The elution mixture (mobile phase) was filtered and degased before using at a flow rate of 1.0 ml/min. Peak areas were recorded and integrated by a Hewlett-Packard 3390-A integrator which was used to quantify chlorpyrifos concentrations in water samples. This procedure follows the method for the determination of chlorpyrifos using high pressure liquid chromatography which was adopted in 1981 (J.Assoc.Anal.Chem. 64: No. 2). The recovery of known concentrations of chlorpyrifos was 78-82% using this method.

2. Residues in fish

At the end of each spawning and growth experiment, fish were removed from tanks, weighed, wrapped in aluminum foil, put in labeled plastic bags, and stored in a freezing room at approximately -20 C. For analysis of chlorpyrifos residues, fish samples were brought from the freezing room, thawed, washed, and scaled off.

For each fish sample, 10 g of fish was blended twice in a Sorvall Omni mixer, after adding 10 g of sodium sulfate anhydrous and 50-ml of acetonitrile. This mixture was filtered under vacuum using Whatman glass filter paper. The extract was then concentrated by rotary evaporation and nitrogen evaporation at 40 C. The residues were dissolved in hexane, then transferred to a 125-ml separatory funnel, and extracted three times with 25-ml portions of acetonitrile. The acetonitrile extracts (the lower layer) were combined and concentrated again as mentioned earlier. The residues were dissolved in two ml acetone for Gas Liquid Chromatography injections. A Beckman GC-65 gas-liquid chromatography equipped with flame photometric detector (phosphorous mode) was used with the following operation conditions: column: J&W 15 meter MegaBore capillary column, DB-5 0.530 mm i.d. and film thickness 1.5 micron, detector temperature : 300 C, oven temperature : 180 C, injection port temperature: 280 C, carrier gas: helium at a flow rate of 25 ml/min., gas flows: air at 200 cc/min. and hydrogen at 150 cc/min.

This method of analysis was developed by Kanazawa (1975). The recovery of known concentrations of chlorpyrifos obtained using this method was 85%.

V. Statistical Analysis and Calculations

For the acute toxicity test, the 96-hr LC50 was calculated using the binomial test method with no partial kills (Stephan, 1977). This method allows calculation of both the LC50 and the confidence level with the following equations:

$$LC50 = (AB)^{1/2}$$

where: A = concentration where no fish die

B = concentration where all fish die

$$\text{Confidence Level} = 100(1 - 2(1/2)^N)$$

where: N = the number of fish exposed

One-way analysis of variance (ANOVA) was used to evaluate differences in chronic effects of chlorpyrifos on spawning and reproductive behavior and early life stage survival of T. zillii. Measures of AchE activity and chlorpyrifos residues also were subjected to ANOVA. Dunnet's method (1955) was then used to compare the treatment means with control means.

The effect of chlorpyrifos on the growth rate of T. zillii was evaluated with the method used by Annet (1985) using the following threshold-corrected hyperbolic

Michaelis-Menten equation:

$$u = u_{\max} \left(\frac{S - S_q}{S + K_s - 2(S_q)} \right)$$

where: u = specific growth rate (1/time)

$u_{\max}$ = maximum specific growth rate (1/time)

S = Substrate food concentration

S_q = threshold (maintenance) level

K_s = substrate food concentration where the specific growth rate is half the maximum specific growth rate "half saturation value"

The specific growth rate (u) in units of 1/day was calculated for fish fed each ration and chlorpyrifos concentrations of zero, 1.7 and 3.6 ug/l with the following equation:

$$u = \frac{\ln W/t_1 - \ln W/t_0}{T}$$

where: WT_0 = initial weight of the fish (grams)

WT_t = weight of the fish at time t
(grams)

T = time (7 days)

The threshold feeding level (S_q), or the feeding level where the fish neither gained nor lost weight, was calculated by performing a linear regression between the values of specific growth rate and the natural logarithm ($\ln$) of the corresponding percent feeding levels (S). A value of one was added to each percent feeding level to

include the data from the fish which were not fed. The resulting equation: $u = a + b \ln (S+1)$ allows calculation of the threshold feeding level (S_q) by solving this equation for S when u equals zero, this being the definition of the threshold feeding level. The other growth constants (K_s and u_{max}) were calculated using a linear transformation of the threshold-corrected hyperbolic equation as follows:

$$\frac{S - S_q}{u} = \frac{K_s - S_q}{u_{max}} + \frac{1}{u_{max}}(S - S_q)$$

Regression was performed between values of $(S - S_q) / u$ and $(S - S_q)$. The reciprocal of the resulting slope was equal to u_{max} . The (K_s) value was obtained by multiplying the intercept $(K_s - S_q / u_{max})$ by the reciprocal of the slope (u_{max}). These growth constants were then used to construct the growth curves for the fish at the control and at both chlorpyrifos concentrations, 1.7 and 3.6 ug/l.

In addition, the "efficiency" of the fish to use the available substrate food concentrations was calculated by dividing the values of u by the corresponding substrate food concentrations (S) and plotting this value (u/S) versus S . These plots establish the "efficiency" curve. That was done for control and both chlorpyrifos concentrations of 1.7 and 3.6 ug/l.

RESULTS

I. Acute Toxicity Test

The acute toxicity of chlorpyrifos was determined using a four day static acute toxicity test. There were no partial kills in any treatment, so the binomial test method was used to calculate a 96-h LC50 of 240 ug/l. The confidence limits of this estimate were 180 and 320 ug/l at a confidence level of 99.80%.

Throughout the acute toxicity test, the fish were observed each day. When chlorpyrifos was introduced into the tanks, fish were very excited, especially at the higher concentrations, and exhibited dark colored bars on their bodies. Some fish came to the surface and remained there for an hour or more. The opercular movements increased and some fish were unable to close their mouths in the last stages before they died. Other fish showed some unusual and rapid movements and swam in circles until they lost their equilibrium. Finally, at the highest concentration of 320 ug/l, some fish swam slowly on their sides on the glass bottom of the tank and settled there until they died. Most of the mortalities at that concentration occurred in the first three days and only one fish died on the fourth and last day of the test. No abnormal movements or evidence of

toxicity were observed at the lowest chlorpyrifos concentration (56 ug/l) or in either water or solvent controls.

II. Chronic Toxicity Tests

1. Spawning and reproductive behavior

In this experiment, the spawning and reproductive behavior of T.zillii was observed under two measured sublethal concentrations of chlorpyrifos, 1.7 and 3.6 ug/L for two spawns (90 days) using the diluter system described in the materials and methods section.

As T.zillii are substrate breeders, females laid their eggs, which are very sticky, in nests on the sandy bottom or on the glass sides of the tank. Both parents then guarded the eggs, fanned them by their pectoral fins, and cleaned them with their mouths. Within two to three days after spawning, the eggs hatched and both parents continued guarding the yolk-sac larvae which formed one or two big clusters on the bottom. After about four days, the larvae yolk sacs were absorbed and larvae began to move in schools accompanied by their guarding parents. The schooling behavior lasted for another six to seven days, during which the relationship between parents and larvae decreased and then ended completely.

This reproductive behavior was successfully observed over two spawns at 1.7 ug chlorpyrifos/l, while this was over one spawn only at 3.6 ug chlorpyrifos/l where the fish could not

spawn for the second time at that concentration. The total period of parental care for eggs and larvae was significantly longer ($P < 0.05$) at 3.6 ug/l than 1.7 ug/l or the control. However, there were no significant differences in the other developmental stages described above between chlorpyrifos concentrations of 1.7 and 3.6 ug/l or the control (Table 3).

2. Early life stages

In this experiment, larvae less than 24 hours old were exposed to two sublethal measured concentrations of chlorpyrifos (1.7 and 3.6 ug/l) for 35 days under the flow-through conditions allowed by the diluter. Mortalities were counted daily and recorded as percent survival in the control and each chlorpyrifos concentration (Table 4). As is shown in Table 4, all 50 fish survived in the control. There was one mortality at 1.7 ug chlorpyrifos/l and fifteen deaths at 3.6 ug/l yielding percent survival of 100, 98, and 70 in the control, 1.7 and 3.6 ug/l respectively. Survival at the 3.6 ug chlorpyrifos/l was significantly lower ($P < 0.05$) than the survival rates obtained at 1.7 ug chlorpyrifos/l or the control.

3. Growth

The specific growth rate (u) for T.zillii at each feeding level and each chlorpyrifos concentration was calculated and is shown in Table 5. The other growth

Table 3. Effect of chlorpyrifos on spawning and reproductive behavior of *T.zillii* exposed to 1.7 and 3.6 ug chlopyrifos/l for 90 days through two spawns.

Measured conc. (ug/l)	Developmental stage (days)			
	Hatching period	Yolk sac absorption	Schooling behavior	Parental care
Control	2.30	4.00	6.00	14.00
1.7	2.50	3.50	6.15	15.00
3.6*	2.70	3.80	6.40	19.00

* Data for one spawn only.

Table 4. Survival of early life stages of T.zillii exposed to 1.7 and 3.6 ug chlorpyrifos/l for 35 days.

Measured conc. (ug/l)	Number of survivals	Survival (%)
Control	50	100
1.7	49	98
3.6	35	70

Table 5. Average specific growth rate (u) of T.zillii fed six levels of Purina Trout Chow (dry weight food/wet fish body weight) and exposed to 1.7 and 3.6 ug chlorpyrifos/l for five weeks.

	Measured conc. (ug/l)	Feeding levels (%)					
		0 % Food	1 % Food	2 % Food	3 % Food	4 % Food	5 % Food
Control	-	0.004	0.010	0.014	0.023	0.023	0.023
1.7	-	0.013	0.008	0.010	0.012	0.020	0.021
3.6	-	0.016	0.007	0.009	0.013	0.018	0.019

constants, the threshold value (S_q), the maximum growth rate (U_{max}), and the half saturation value (K_s), were calculated and are given in Table 6. These kinetic growth constants allowed construction of the growth curves shown in Figure 2 for the control and the two chlorpyrifos concentrations. As shown in Table 6 and Figures 3, 4, and 5, the S_q values, the threshold level, increased with increased chlorpyrifos concentrations and the values for u_{max} and K_s decreased with increased chlorpyrifos concentrations.

The efficiency (u/S) was calculated for the fish at the control and both chlorpyrifos concentrations and efficiency curves are given in Figure 6.

III. Brain Acetylcholinesterase (AChE) Activity

1. Adults exposed for 90 days

After adult males and females were exposed to two sublethal concentrations of chlorpyrifos of 1.7 and 3.6 $\mu\text{g/l}$ for 90 days in the spawning experiment, the brain AChE activity was measured colorimetrically (Ellman et. al., 1961), and values are shown in Table 7. The AChE activity at both chlorpyrifos concentrations was significantly less ($P < 0.05$) than the control, and AChE inhibition was significantly greater at a concentration of 3.6 $\mu\text{g/l}$ than at 1.7 $\mu\text{g/l}$ (Table 7). In addition, the reduction in AChE activity in females was significantly greater ($P < 0.05$) than that observed for the males exposed to both concentrations of

Table 6. Kinetic growth constants of T.zillii fed Purina Trout Chow and exposed to 1.7 and 3.6 ug chlorpyrifos/l.

Measured conc. (ug/l)	Kinetic growth constants		
	Sq	umax	Ks
	(g dry food/ g wet weight fish/ day)	(1/days)	(g dry food/ g wet weight fish/ day)
Control	0.269	0.036	3.272
1.7	0.733	0.027	2.244
3.6	0.941	0.022	1.813

Figure 2. Effect of chlorpyrifos on the specific growth rate (μ) of T.zillii exposed to 1.7 and 3.6 ug chlorpyrifos/l for 35 days.

Figure 2.

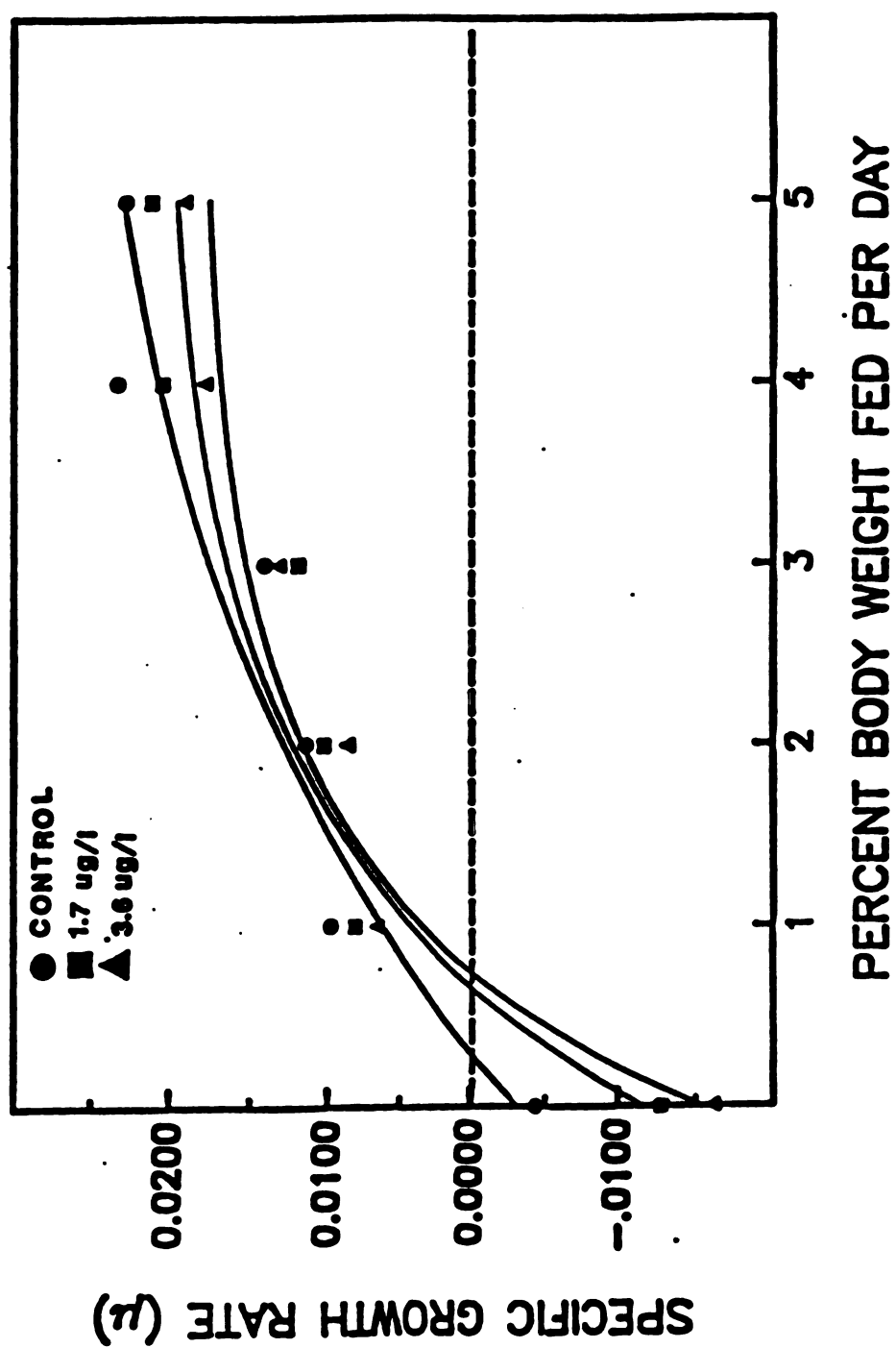


Figure 3. Effect of chlorpyrifos on the half saturation value (K_s) of T.zillii after exposure to 1.7 and 3.6 ug chlorpyrifos /l for 35 days.

Figure 3.

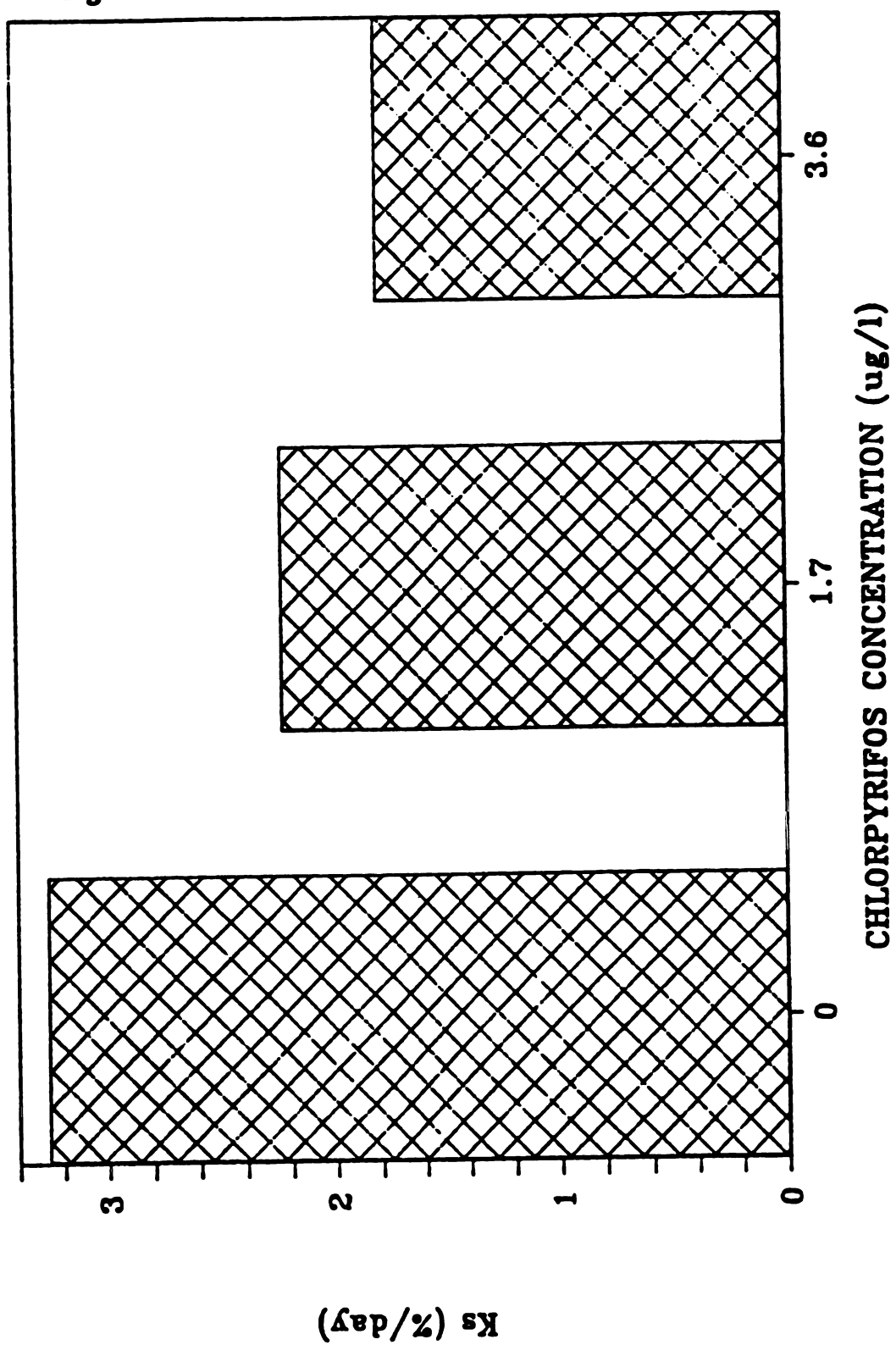


Figure 4. Effect of chlorpyrifos on the maximum specific growth rate ($\mu_{\max}$) of T.zillii after exposure to 1.7 and 3.6 ug chlorpyrifos/l for 35. days.

Figure 4.

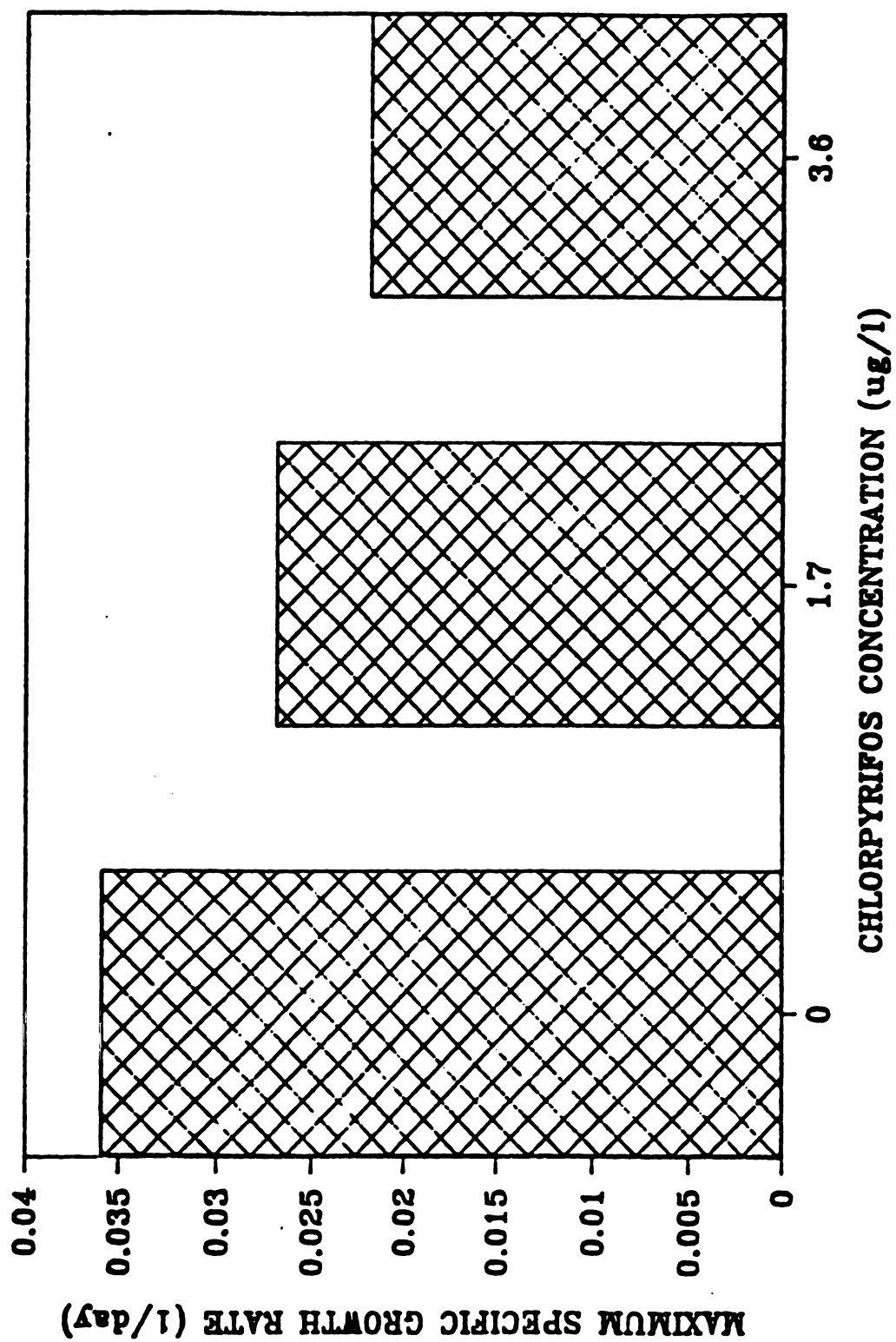


Figure 5. Effect of chlorpyrifos on the threshold value (Sq) of T.zillii after exposure to 1.7 and 3.6 ug chlorpyrifos/l for 35 days.

Figure 5.

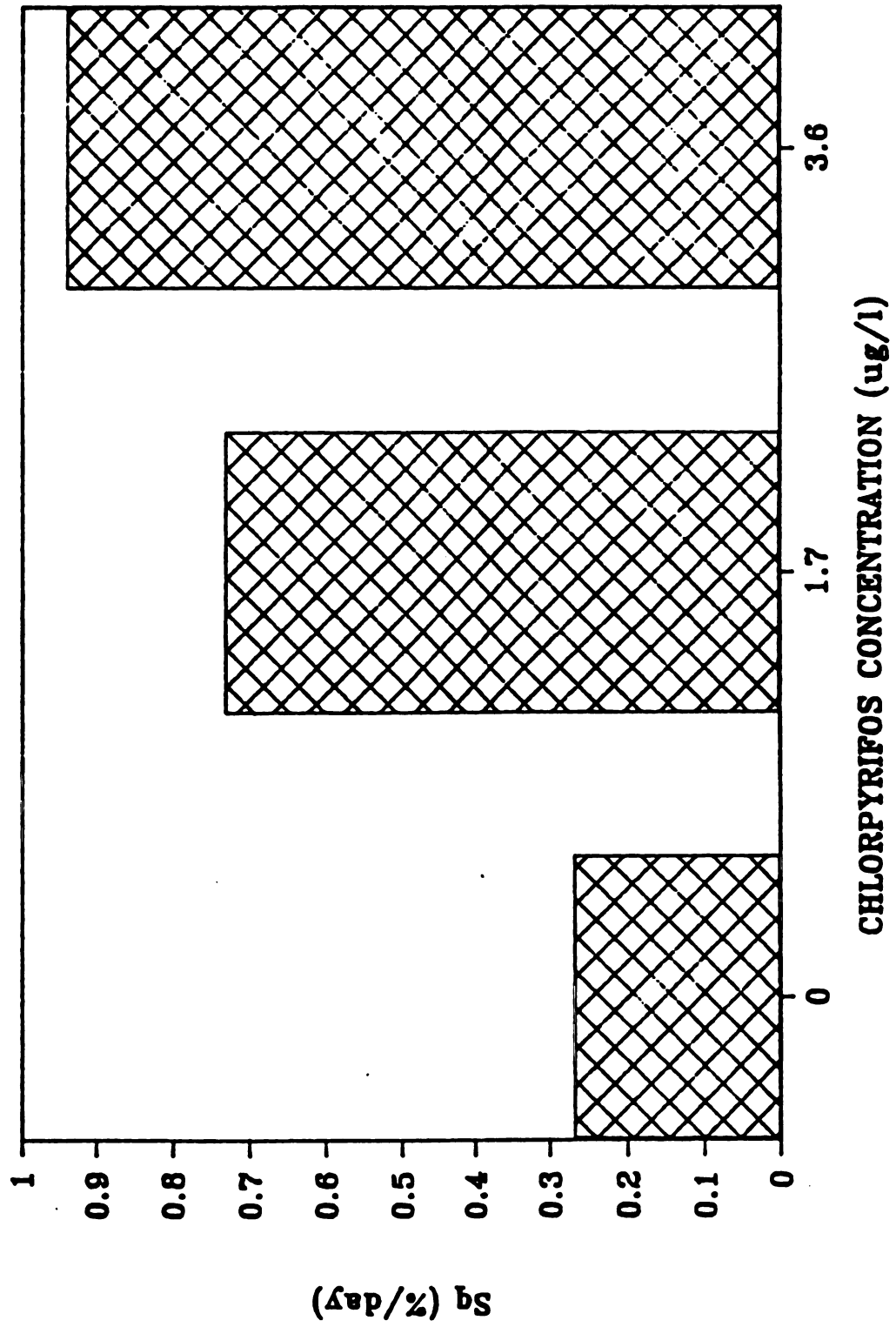


Figure 6. Effect of chlorpyrifos on the efficiency of T.zillii after exposure to 1.7 and 3.6 ug chlorpyrifos/l for 35 days.

Figure 6.

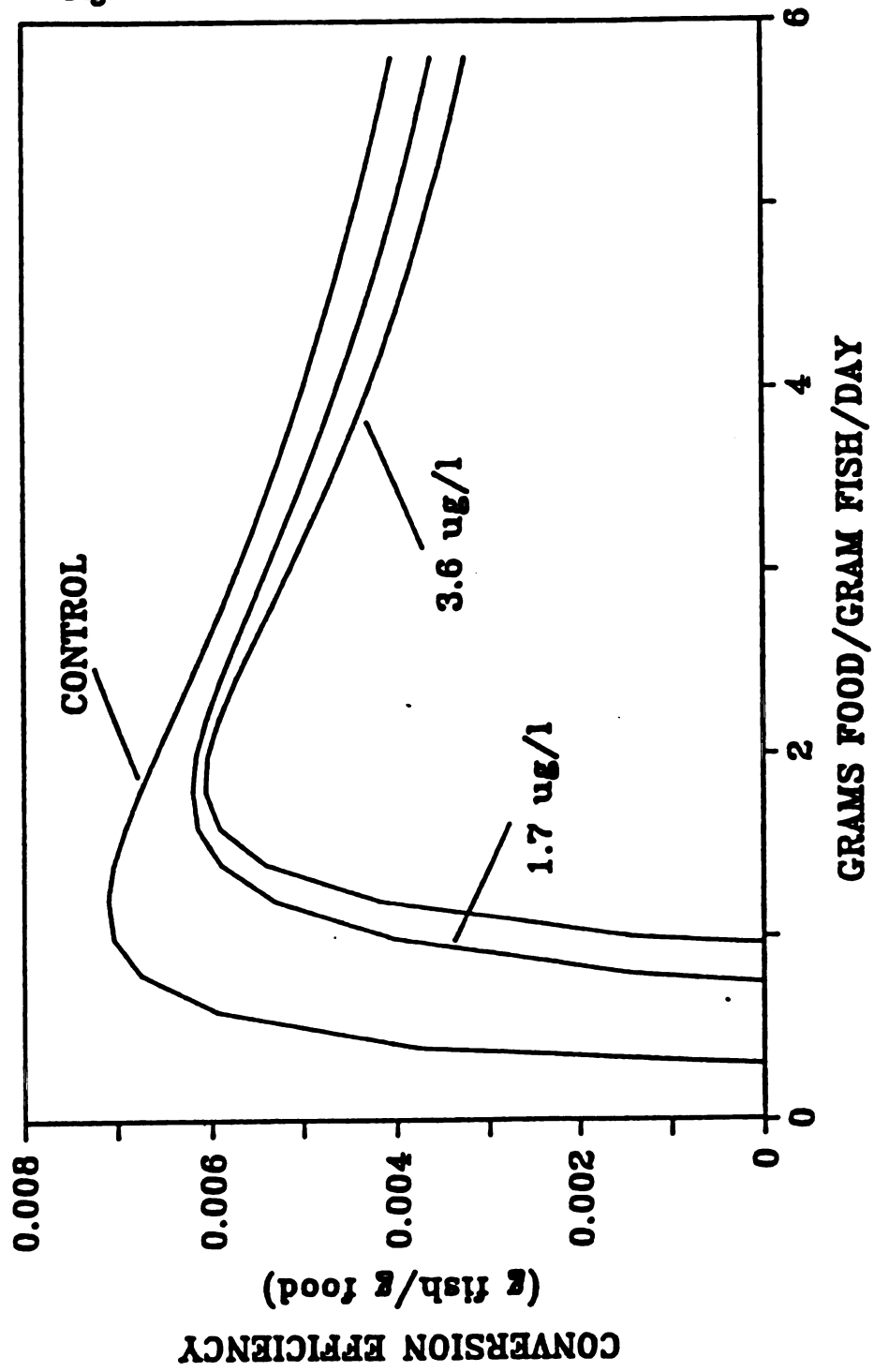


Table 7. Brain acetylcholinesterase (AChE) activity and percent inhibition of AChE in T.zillii adult females and males after exposure to 1.7 and 3.6 ug chlorpyrifos/l for 90 days. AChE is expressed as umoles/mg protein/minute.

<u>Measured conc. (ug/l)</u>	<u>Females</u>	<u>Males</u>
<u>Control</u>		
AChE activity	285.90	290.10
% inhib.	00.00	00.00
<u>1.7</u>		
AChE activity	80.70	101.00
% inhib.	71.00	65.18
<u>3.6</u>		
AChE activity	57.40	78.90
% inhib.	79.90	72.80

chlorpyrifos. For females, the percent inhibition of AchE reached 71 and 79.90 percent, while it was 65.18 and 72.80 percent for males at 1.7 and 3.6 ug/l respectively.

2. Fingerlings exposed for 35 days

At the end of the 35 day exposure period in the growth experiment, AchE activity in the brains of the fingerling T.zillii was analyzed for fish fed at six feeding levels, 0%, 1%, 2%, 3%, 4%, and 5% of the wet body weight for the control and for each concentration of chlorpyrifos used as is shown in Table 8. The percent AchE inhibition ranged from 49-56 percent at 1.7 ug/l chlorpyrifos and from 59-67 percent at 3.6 ug/l. Significant differences were observed in both AchE activity and percent inhibition for control and both concentrations of chlorpyrifos ($P < 0.05$) at all feeding levels. However, there was no significant differences in either AchE activity or percent inhibition of AchE as a function of feeding level at either concentration.

IV. Chlorpyrifos Residues in Tissues and Bioconcentration Factor

1. Adults exposed for 90 days

Chlorpyrifos residues in the muscle tissues of T.zillii adults exposed to chlorpyrifos for 90 days in the spawning experiment were analyzed. These residues were significantly ($P < 0.05$) greater in fish exposed to 3.6 ug chlorpyrifos/l than at 1.7 ug chlorpyrifos/l as is given in Table 9.

Table 8. Brain acetylcholinesterase (AChE) activity and percent inhibition AChE of *T.zillii* fingerlings after exposure to 1.7 and 3.6 ug chlorpyrifos/l for 35 days and fed Purina Trout Chow at six different feeding levels (dry weight food/wet fish body weight). AChE is expressed as umoles/mg protein/minute.

<u>Measured conc. (ug/l)</u>	<u>Feeding levels (%)</u>					
	<u>0%</u> Food	<u>1%</u> Food	<u>2%</u> Food	<u>3%</u> Food	<u>4%</u> Food	<u>5%</u> Food
<u>Control</u>						
AChE activity	280.11	278.04	271.32	280.12	279.12	277.09
% inhib.	00.00	00.00	00.00	00.00	00.00	00.00
<u>1.7</u>						
AChE activity	140.04	137.72	133.39	143.58	124.52	121.70
% inhib.	50.00	50.47	50.84	48.74	55.39	56.08
<u>3.6</u>						
AChE activity	115.94	112.93	109.53	108.01	120.06	105.12
% inhib.	58.61	60.10	61.11	62.87	67.38	63.87

Table 9. Tissue residues of chlorpyrifos (mg/kg) and bioconcentration factor (BCF) in T.zillii females and males, following exposure to 1.7 and ug chlorpyrifos/l for 90 days.

Measured conc. (ug/l)	Females	Males
<u>Control</u>		
Residues (mg/kg)	ND*	ND
BCF	---	---
<u>1.7</u>		
Residues (mg/kg)	0.520	0.344
BCF	306	202
<u>3.6</u>		
Residues (mg/kg)	0.920	0.850
BCF	541	500

*** Not detected.**

However, there was no significant difference between males and females in the chlorpyrifos residues content at either chlorpyrifos concentrations of 1.7 and 3.6 ug/l.

The bioconcentration factor (BCF), which is defined as the ratio between concentration of chlorpyrifos in tissues and concentration of chlorpyrifos in water at equilibrium, was calculated to be 306 and 202 for females and males respectively at 1.7 ug chlorpyrifos/l while it was 541 and 500 for females and males respectively at 3.6 ug chlorpyrifos/l as given in Table 9.

2. Fingerlings exposed for 35 days

The chlorpyrifos residue content of the tissues of fingerlings ranged from 0.205 to 0.403 mg/kg at 1.7 ug chlorpyrifos/l and ranged from 0.311 to 0.901 mg/Kg at 3.6 ug/l. Again, the residue content was significantly greater ($P < 0.05$) at 3.6 ug/l than at 1.7 ug/l chlorpyrifos. However there was no significant relationship in the chlorpyrifos residue content of the fingerlings at the six different feeding levels (Table 10).

Table 10. Tissue residues of chlorpyrifos (mg/kg) and bioconcentration factor (BCF) in *T.zillii* fingerlings following exposure to 1.7 and 3.6 ug chlorpyrifos/l for 35 days fed Purina Trout Chow at six feeding levels.

**Measured
conc.
(ug/l)**

Feeding levels (%)

	0% Food	1% Food	2% Food	3% Food	4% Food	5% Food
--	------------	------------	------------	------------	------------	------------

Control

Residues (mg/kg)	ND*	ND	ND	ND	ND	ND
-----------------------------	-----	----	----	----	----	----

BCF	---	---	---	---	---	---
------------	-----	-----	-----	-----	-----	-----

1.7

Residues (mg/kg)	0.205	0.403	0.198	0.209	0.305	0.335
-----------------------------	-------	-------	-------	-------	-------	-------

BCF	121	237	116	123	179	197
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3.6

Residues (mg/kg)	0.509	0.611	0.901	0.690	0.794	0.670
-----------------------------	-------	-------	-------	-------	-------	-------

BCF	141	170	250	192	221	186
------------	-----	-----	-----	-----	-----	-----

* Not detected.

DISCUSSION

The expanding use of an ever increasing variety of pesticides to control agricultural insects and pests throughout the world is accompanied by increases in the potential contamination of water by these pesticides. Effluent discharge from pesticide manufacturing plants and accidental spills of pesticides are obvious point sources of water pollution by pesticides. Aerial drift and runoff from agricultural application are considered to be the main nonpoint sources of water pollution by pesticides (Nicholson, 1969). In addition, careless washing and cleaning of pesticide equipment and containers is another source of contaminating water by pesticides. However, nonpoint sources of water pollution by pesticides are much more difficult to detect and control than those from point sources. Pesticide legislation acts, economic pressure, and adverse publicity can affect those pesticide companies that continue to pollute water from point sources, but it is much more difficult control nonpoint sources (Egner et. al., 1983; United States Environmental Protection Agency, 1985).

Depending on the concentration, the addition of pesticides to the aquatic environment could be lethal to aquatic organisms; or at lower concentrations could have a long term chronic effect on such organisms. The increasing

awareness of the environmental hazards of pesticides has resulted in the development of a variety of methods to evaluate the toxicity of pesticides to aquatic organisms (Murty, 1986). Such tests are to detect and evaluate the potential toxicological effects of chemicals, including pesticides, on fish and aquatic organisms.

There are different types of toxicity tests which serve different purposes in evaluating the impact of pesticides on fish. The type of test procedure required to give a measure of the acute lethal concentration for fish is called the short term or acute toxicity test and is one of the most commonly used methods in the evaluation of such toxicity. The common result of acute toxicity tests is the determination of the concentration of the toxicant which will kill one half of the fish in a short time period (24, 48, 72, or 96 hours).

Another approach is to use chronic tests in which the fish are exposed to very low concentrations (sublethal levels) which may not directly cause death but may cause some adverse effects on the spawning, survival, growth, or the behavior of the fish. Such effects could go unnoticed in the acute toxicity tests (Rand, 1980). Chronic tests allow a better chance of determining safe levels for the chemicals under investigation so that the application factors can be calculated.

In this study, toxicity tests were conducted to evaluate the different adverse effects of the organophosphate

insecticide chlorpyrifos on the fish T.zillii. The static 96-h acute toxicity test was chosen as a rapid, simple, and inexpensive procedure to evaluate the concentration of chlorpyrifos that kills 50% of the fish population within 96 hours. The chronic effect of chlorpyrifos on spawning and reproductive behavior, survival of early life stages, and growth of the fish then was evaluated through different standardized chronic tests.

I. Acute Toxicity Study

The acute toxicity test provides useful information about the concentration of a toxicant which can cause adverse effects, mostly lethal, to the test organism. These tests also can serve in evaluating the relative toxicity of a large number of toxicants and in determining the relative sensitivity of organisms to a single toxicant or to mixtures of toxicants. The commonly used term, LC50, means that concentration which kills 50 percent of the test organisms during a continuous exposure at a specific period of time, 24, 48, 72, or 96 h, (United States Environmental Protection Agency, 1982). The use of the 50 percent mortality level is based on the fact that at this point results are more consistent and less variable than at high or low mortality levels, i.e., 100% and 0% mortality (Muirhead-Thomson, 1971). In this study, death was used as a measure of chlorpyrifos acute toxicity to T.zillii and the exposure period was 96 h.

In this investigation, a static acute toxicity test was conducted to evaluate the 96-h LC50 concentration of chlorpyrifos for T.zillii. Since there were no partial kills during the test, the binomial test method was used to estimate the 96-h LC50 (Stephan, 1977). The 96-h LC50 for T.zillii subjected to chlorpyrifos was calculated to be 240 ug/l. Although the binomial test usually estimates an LC50 comparable to a probit or moving average, its wider more conservative confidence interval is not based on the 95% confidence limits (Borthwick et al., 1985). The level of confidence is usually above 95% if six or more organisms are exposed in each concentration tested (Stephan, 1977). In this study, the confidence limits for the calculated 96-h LC50 (240 ug/l) were 180 and 320 ug/L and the confidence level was calculated as 99.80%.

A comparison of the acute toxicity of chlorpyrifos for T.zillii with other fish species is given in Table 11. The comparison data in Table 11 is compiled from Marshall and Roberts, 1978; United States Environmental Protection Agency, 1986; Odenkirchen and Eisler, 1988. From Table 11, it is apparent that the goldfish, Carassius auratus; the channel catfish, Ictalurus punctatus; the gulf toadfish, Opsanus beta; and the mosquitofish, Gambusia affinis can survive relatively high levels of chlorpyrifos. On the other hand, the most sensitive fish to chlorpyrifos is the california grunion, Leuresthes tenuis, which has 96-h LC50 value of 1.3 ug/l, followed in sensitivity by the bluegill,

Table 11. Acute toxicity of chlorpyrifos to different fish species. Data are compiled from Marshall and Roberts (1978); United States Environmental Protection Agency (1986); Odenkirchen and Eisler (1988).

Fish species	Method*	LC50** (ug/l)	Reference
Rainbow trout (<u>Salmo gairdneri</u>)	S,U	7.1	Macek et. al., (1969)
Bluegill (<u>Lepomis macrochirus</u>)	S,U	2.4	Johnson and Finely (1980)
Fathead minnow (<u>Pimephales promelas</u>)	S,M	170.0	Jarvinen and Tanner (1982)
Lake trout (<u>Salvelinus nanaycush</u>)	S,U	98.0	Johnson and Finely (1980)
Mosquitofish (<u>Gambusia affinis</u>)	S,U	280.0	Carter and Graves (1973)
Channel catfish (<u>Ictalurus punctatus</u>)	F,M	280.0	Johnson and Finley (1980)
Goldfish (<u>Carassius auratus</u>)	S,U	180.0***	Kenaga et. al., (1965)
Sheephead minnow (<u>Cyprinodon variegatus</u>)	S,U	270.0	Borthwick and Walsh (1981)

Table 11. Cont'd.

Gulf toadfish (<u>Opsanus beta</u>)	R,M	520.0	Hansen et. al., (1986)
California grunion (<u>Leuresthes tenuis</u>)	F,M	1.3	Borthwick et. al., (1985)
Inland silverside (<u>Menidia beryllina</u>)	F,M	4.2	Clark et. al., (1985)
Striped mullet (<u>Mugil cephalus</u>)	F,M	5.4	Schimmel et. al., (1983)
<u>Tilapia zillii</u>	S,U	240.0	(This study)

* S: static

R: renewal

F: flow through

M: concentration measured during test

U: concentration not measured during test

** 96-h LC50

*** 24-h LC50

Lepomis macrochirus, which has a 96-h LC50 of 2.4 ug/l determined in a static and unmeasured acute test (Table 11). With its 96-h LC50 of 240 ug/l, T.zillii is considered to be among the more insensitive fish species to chlorpyrifos.

However, few chlorpyrifos studies have been conducted with tilapia species. El-Refai et. al., (1976) estimated the 24-h LC50 for Oreochromis niloticus to be 139 ug/l in a static acute test. In another static acute test, Herzberg (1987) reported the 72-h LC50 for O.aureus to be in the range of 151-191 ug/l and the 24-hr LC50 for O.niloticus was estimated as 418 ug/l. The variations in results on tilapia species on their reaction to chlorpyrifos could be related to the test fish species itself. Specific differences between closely related tilapia species in their reactions to toxicants were reported in some other studies (Muirhead-Thomson, 1971). When a flowing water was treated with the molluscicide, Tritylmorpholine, the fish, O.mossambicus survived seven days, while under the same conditions, Sarotherodon melanotheron died. Also, T.parmanii was much more tolerant than the other two species, S.melanotherodon and S.macrochir on their reactions to dieldrin treatment (Muirhead-Thomson, 1971). Other possible reasons for the variability in tilapia species acute response to chlorpyrifos are, the chemical formulation used in the test, water temperature during the test, or varying chemical characteristics of water used in the test (i.e., alkalinity, hardness, and pH).

II. Chronic Toxicity Studies

The primary value from conducting the chronic toxicity tests is to determine the "safe" levels of toxicants (Brown, 1973), which can not be detected using the acute toxicity tests. Therefore, these tests are currently much more valuable than the other acute tests.

In the present study, the spawning and reproductive behavior, survival of early life stages, and growth of T.zillii were tested with two sublethal concentrations of chlorpyrifos, 1.7 and 3.6 ug/l using the proportional diluter system (Mount and Brungs, 1967; Lemke et. al., 1978) described in the materials and methods section.

1. Spawning and reproductive behavior

Many investigators have studied the breeding and reproductive behavior of T.zillii in the field or under laboratory conditions (EL-Zarka, 1956; Imam and Hashem, 1966; Loiselle, 1977; Siddiqui, 1979a; Rothbard, 1979). However, there is no known published study about the effect of any kind of pesticides on the reproductive behavior of the fish.

In this investigation, the spawning and reproductive behavior of T.zillii subjected to chlorpyrifos was studied using the continuous flow-through exposure of 1.7 and 3.6 ug /l. Since the fish used in this experiment were ripe females and mature males, eggs were produced within a few

days after the fish were placed in the aquaria. Since the short exposure time before the first spawning was not long enough to determine the sublethal effect of chlorpyrifos, the fish were retained in the aquaria until they spawned a second time. During the second spawn, eggs were produced normally at the control and 1.7 ug/l, but no eggs were produced at 3.6 ug chlorpyrifos/l. The only significant observed difference in T.zillii reproductive behavior was in the length of parental care period for eggs and young. Parental care lasted significantly longer ($P < 0.05$) at 3.6 ug chlorpyrifos/l than at 1.7 ug chlorpyrifos/l (Table 3). The failure of the fish to spawn at 3.6 ug chlorpyrifos/l for a second time negated any possibility of determining any significant difference in the other developmental stages shown in Table 3.

Jarvinen et. al., (1983) studied the chronic effect of the encapsulated slow-release formulation of chlorpyrifos on fathead minnows for 200 days including a reproductive period in the life cycle of the fish. The mean number of spawns per spawning pair of the fathead minnows was significantly reduced at 2.68 ug chlorpyrifos/l and the total egg production at 0.63 ug chlorpyrifos/l and higher was significantly lower than in the controls.

In another study (Adelman et. al., 1976), fathead minnows were exposed to another organophosphate insecticide, Guthion, over a complete life cycle. These authors reported that the number of eggs per spawning and per female was

reduced at concentrations as low as 0.51 ug Guthion/l.

When the same fish, fathead minnows, were exposed continuously to four sublethal concentrations of the organophosphorus insecticide malathion, the sex ratios and number of spawnings per female were significantly reduced at concentrations as low as 580 ug malathion/l (Mount and Stephan, 1967). Malathion, at concentrations greater than 20 ug/l, had another marked effect on the number of spawns and the number of eggs produced per spawn by bluegills (Eaton, 1970). Carlson (1972) found that a concentration of 680 ug/L of carbaryl (Sevin), a carbamate insecticide, adversely affected the spawning of fathead minnows. At this concentration, the mean number of eggs produced per female and the mean number of eggs per spawn were less than the control and other lower concentrations.

Yasuno et. al., (1980) reported that the organophosphate insecticide, temphos reduced the normal birth of the viviparous guppy, Poecilia reticulata, one month after exposure to 1500 ug temphos/l, while with fenithrothion, the number of young produced decreased at 250 ug/l, 500 ug/l, and 1.0 mg/l during the second month of exposure.

In the cichlid fish, O.mossambicus, Matthiessen and Logan (1984) found that the organochlorine insecticide, endosulfon, ended the sexual behavior of the fish at 0.6 ug/l and the dosed males were abnormally sensitive to any stimuli. In my study, T.zillii did not spawn a second time at a chlorpyrifos concentration of 3.6 ug/l.

2. Early life stages

The use of early life stage (ELS) toxicity tests has been proposed by several investigators as a means of evaluating the sublethal effect of many pollutants on fish (Pickering and Thatcher, 1970; Pickering and cast, 1972; Mckim et. al., 1975 & 1978; Mckim, 1977; Sauter et. al., 1976; Macek and Sleight, 1977; Eaton et. al., 1978; Beniot et. al., 1982).

In an extensive review of the complete and partial life cycle toxicity tests, Macek and Sleight (1977) concluded that exposure of the critical life stages, early life stages, can provide good estimates of the "safe" concentrations very similar to those derived from complete life cycle chronic toxicity tests. The data obtained from such early life stage tests are also very useful in the evaluation of total hazard assessment scheme (Cairns et. al., 1978).

According to my findings for the early life stage study of T.zillii with chlorpyrifos, the lower chronic value or the no observed effect level (NOEL), which is defined as the highest concentration that has no statistically adverse effect different from the control, was 1.7 ug chlorpyrifos/L for these young fish. The upper chronic value or the lowest observed effect level (LOEL), which is defined as the lowest concentration which causes a statistically adverse effect different from the control, was 3.6 ug chlorpyrifos/l.

Accordingly, the maximum acceptable toxixant

concentration (MATC) or the "no effect" level, which is defined as the hypothetical toxic threshold concentration between the lower chronic value NOEL and the upper chronic value LOEL (Mount and Stephan, 1967) is between 1.7 and 3.6 ug/l.

When the MATC values, estimated from the early life stages, were compared with MATC values calculated from the longer chronic toxicity tests, 82% of the time they were found to be identical (Mckim, 1977). He added that water quality, species, type of toxicant, and parental exposure had little effect on the estimation of MATC values. However, Beniot et. al., (1982) suggested that if MATC is calculated from chronic tests (partial life cycle or early life stages), rather than the complete life cycle toxicity tests, MATC should be redefined as an "estimated MATC".

The "chronic value" can be calculated by taking the geometric mean of the lower and upper chronic values (United States Environmental Protection Agency, 1985). In my study, this chronic value was estimated as 2.474 ug chlorpyrifos/l for T.zillii.

Jarvinen and Tanner (1982) in their studies with the technical grade and encapsulated formulations of chlorpyrifos, found a significant reduction in survival of fathead minnows at 3.2 ug/l and 4.8 ug/l of the chlorpyrifos technical grade and encapsulated formulation respectively. Also, Jarvinen et. al., (1983) observed similar reduction in survival of fathead minnows when fish were exposed to the

encapsulated formulation of chlorpyrifos at 2.68 ug/l for 60 days. Goodman et. al., (1985b) conducted 28 day early life stage chronic toxicity tests using three estuarine species of atherinid fishes with chlorpyrifos. The concentrations that caused significant reduction in survival of the hatched fish were : 1.8 ug/l for inland silverside (Menidi beryllina), 0.48 ug/l for the atlantic silverside (M. menidia), and 0.78 ug/l for the tide-water silverside (M. peninsulae). Early life stages of gulf toadfish (Opsanus beta) were exposed to chlorpyrifos in two 49-day toxicity tests (Hansen et. al., 1986), and a significant reduction in fish survival was observed only at a concentration as high as 150 ug/l. However the toadfish larvae weighed significantly less than the control at concentration as low as 3.7 ug/l. The survival of fathead minnow larvae, produced by unexposed parents, was significantly reduced at 680 ug/l of the carbamate insecticide carbaryl (sevin), (Carlson, 1972). When the chronic values obtained in this study are compared with other fish species in Table 12, again, T.zillii could be considered among the more insensitive fish species to chlorpyrifos.

The application factor (AF) is calculated by dividing the "estimated MATC" by the 96-h LC50 (Mount and Stephan, 1967). This application factor is usually reported as a unitless value and a range between the numerical value resulting from dividing the lower and upper limits of (MATC) by the 96-h LC50. Based on these considerations, the

Table 12. Chronic toxicity values of chlorpyrifos for different fish species. These values were estimated from early life stage toxicity tests (after United States Environmental Protection Agency, 1986).

Fish species	Lower & upper chronic values (ug/l)	Chronic value (ug/l)	Application factor	Reference
Fathead minnow	1.6-3.2	2.263	0.009-0.019	(1)
Gulf toafish	1.4-3.7	2.276	0.003-0.007	(2)
Sheephead minnow	1.7-3.0	2.258	0.006-0.011	(3)
Clifornia grunion	0.14-0.30	0.205	0.108-0.231	(4)
Inland silverside	0.75-1.8	1.62	0.179-0.429	(5)
<u>Tilapia zillii</u>	1.7-3.6	2.474	0.007-0.015	(6)

(1) Jarvinen and Tanner, (1982).

(2) Hansen et. al., (1986).

(3) Cripe et. al., (1986).

(4) Goodman et. al., (1985a).

(5) Goodman et. al., (1985b).

(6) This study.

application factor for chlorpyrifos and T.zillii is between 0.007 and 0.015.

Historically, Henderson and Pickering (1957) suggested using a value of 0.1 of the 96-h LC50 as a safe or application value. Other general application factors (i.e., 0.05, 0.01) have been used in conjunction with acute toxic data (Macek and Sleight, 1977), until Mount and Stephan (1967) developed a method to estimate the application factor value based on the relationship between the acute and chronic toxicity of a chemical to a species. Mount (1977) concluded that application factor is a constant and characteristic for the toxicant and could be applied for most or all fish species and water types. He also stated that " the objective in using an AF approach is to integrate effects of variable species sensitivity, length of exposure and effect of water characteristics on toxicity, and to enable one to estimate acceptable concentrations without long expensive tests on a large number of species and waters". However, he questioned the utility of application factors after finding some practical problems with some data for establishing application factors for some chemicals in different water types and for different species, so he concluded that a better method to predict acceptable concentration is needed.

3. Growth

Growth may be defined as "the process of increase or the progressive development of an organism, and may be measured by the change in length or weight of an individual fish or a group of fish species between two sampling times" (Everhart and Youngs, 1981). The growth of fish is affected by many factors, and food quantity and availability is one of the most important factors. In fact, limited food availability will result in fish growth rate far below the maximum potential (Bowen, 1982). Temperature, oxygen, alkalinity, and other water quality factors are considered other common determinants to the growth of the fish (Everhart and Youngs, 1981).

In addition, growth of fish was found to be highly affected and suppressed by the presence of even very low concentrations of pollutants in aquatic ecosystem (Pickering and Thatcher, 1970; Jarvinen and Tanner, 1982; Beniot et. al., 1982; Norberg and Mount, 1985; Cripe et. al., 1986). These investigators concluded that growth of the fish is the most sensitive parameter measured for the different toxic compounds they used in their studies.

The adverse effect of chlorpyrifos on the growth rate of T.zillii was related mainly to the food rations used in this study (0%, 1%, 2%, 3%, 4%, and 5% of the wet body weight) and the concentration of the insecticide chlorpyrifos itself (1.7 and 3.6 ug/l). At the same time, while the calculated

specific growth rate (u), maximum growth rate (u_{max}), and half saturation value (K_s) of T.zillii were reduced at both chlorpyrifos concentrations (1.7 and 3.6 ug/l) the threshold value (S_q) of the fish increased at the same chlorpyrifos concentrations (Table 4 and Figures 3, 4, and 5).

Norberg and Mount (1985) proposed a 7-day subchronic growth test as a short and rapid method to estimate the chronic toxicity of effluents to fish. They also reported that this test can be used to estimate the values of NOEC. In their studies, Dursban (chlorpyrifos) was used as a single toxicant with fathead minnow larvae, and growth was reduced significantly at 7.4 ug/L (7-day renewal test). Jarvinen et. al., (1983) found that growth of fathead minnows was reduced at 2.68 ug/l within 30 days and at 1.21 ug/l by 60 days of exposing to chlorpyrifos. In the same study, the authors noticed that growth and estimated biomass of 30-day-old second generation fish were significantly reduced at 0.12 ug/l which was the lowest concentration tested in their studies. Again, Hansen et. al., (1986) concluded that growth is a much more sensitive endpoint to toxicants than survival of the gulf toadfish, Opsanus beta. This conclusion was made through two early life stage toxicity tests for gulf toadfish with chlorpyrifos to evaluate using this fish as a bioassay organism in toxicity tests. The same conclusion was found in my study when the growth rate of T.zillii was reduced at a concentration as small as 1.7 ug chlorpyrifos/l (Table 3 and Figure 2).

Cripe et. al., (1986) studied the influence of feeding rates on the toxicity of chlorpyrifos to sheephead minnows (Cyprinodon variegatus) during early life stage toxicity tests. Growth was significantly reduced at each chlorpyrifos concentration (3.1 to 52.00 ug/l) and at all feeding levels. Also, they concluded that there was a relationship between the feeding levels and the growth rate of sheephead minnows, which agrees with my findings where the food ration was the most important factor for the growth rate of T.zillii.

McCracken and Leduc (1980) found that the toxicity of cyanide to juvenile rainbow trout, held in swimming chambers during 20-day growth experiments, increased with the increase of fish size and food ration. Also, they reported that cyanide increased the food maintenance requirement of the fish two to three times the control, based on the dry weight gain. They related such increase in food maintenance to the increase in cyanide concentration in growth chambers, which agrees with my findings in this study where the food maintenance value (Sq) of T.zillii increased with the increase of chlorpyrifos concentration (Table 6).

In a recent study, Siefert et. al., (1988) have evaluated the toxicity of chlorpyrifos to fathead minnows in littoral enclosures and found that the mean weight and lengths of fish from the treated enclosures were significantly lower than those in the control. They noticed that the most dramatic effect on growth was during the first two weeks.

III. Brain Acetylcholinesterase (AChE) Activity

The mode of action of all organophosphorus compounds is mainly due to their ability to adversely inhibit the enzyme acetylcholinesterase, AChE (Eto, 1973). This AChE inhibition in animals, including fish, has been demonstrated many times both in vitro and in vivo.

Studies by Weiss in 1958, 1959, and 1961 showed that fish exposed to various lethal and sublethal levels of several organophosphate compounds exhibited a reduced level of AChE activity in the excised brain tissues. He also indicated that death of several species of freshwater fish occurred when brain AChE activity is reduced to 40-70% of that of nonexposed control fish of the same species. In addition, he suggested that brain AChE inhibition in fish could be used as a good tool for detecting the presence of organophosphorus pollutants in natural waters. The recovery to normal AChE activities after exposure to organophosphorus insecticides may take a month or more; therefore it may be possible to detect not only present but also past intoxication (Weiss, 1961; Williams and Sova, 1966).

The insecticide chlorpyrifos, as being one of the organophosphorus compounds, exerts its toxicity by inhibiting the activity of the enzyme, AChE. The oxygen analog of chlorpyrifos is the most potent inhibitor of AChE, about 18,000 times more inhibitory than the parent compound

(Thirugnanam and Furgash, 1977).

Macek et. al., (1972) showed that in ponds treated with chlorpyrifos at 0.05 lb./A, fish exhibited more than 80% inhibition of brain AchE as compared to fish from untreated ponds. Fish from these treated ponds had not recovered normal AchE activity 28 days after application. After a second application of 0.05 lb./A of chlorpyrifos to these ponds, the fish showed an increase in their AchE inhibition which could be a result of the increased rate of conversion of P=S phosphorothioate to the P=O analog at the higher temperature of the ponds.

Thirugnanam and Furgash (1977) conducted in vitro and in vivo studies on the inhibition of anticholinesterase activity of chlorpyrifos on the fish, Fundulus heteroclitus, and found that chlorpyrifos is extremely toxic, the AchE inhibition increased over time and recovery was very slow under field conditions. In the 200-day chronic studies conducted by Jarvinen et. al., (1983) with chlorpyrifos and fathead minnows, the levels of AchE inhibition increased with increased chlorpyrifos concentration in water. The percentage of inhibition ranged from 0 to 10% at 0.12 ug/l, 21 to 41% at 0.27 ug/l, 59% at 0.63 ug/l, 65 to 80% at 1.21 ug/l, and 77 to 89% at 2.68 ug/l.

In my study, the brain AchE activity of T.zillii was significantly lower at both concentrations of chlorpyrifos, (1.7 and 3.6 ug/l) than in the control for both adults (exposed for 90 days) and fingerlings (exposed for 35 days),

(Tables 7 and 8). In addition, the brain AchE inhibition was significantly ($P < 0.05$) greater in females than in males, (Table 7), which perhaps reflects the presence of more fat tissues in females than males. Fats even in small amounts, can make some differences in the inhibition of AchE between females and males (Marshall and Roberts, 1978). However, the AchE activity did not vary significantly as a function of amount of food fed to the fingerlings in the growth experiments (Table 8).

In the spawning and reproductive behavior study, the fish could not spawn for a second time at the 3.6 ug/l concentration, perhaps because of AchE inhibition. Goodman et.al., (1979) suggested the same conclusion when he found impaired reproduction in sheephead minnows associated with acetylcholinesterase inhibition of 27% which occurred when fish were exposed continuously to another organophosphorus, Diazinon.

Urmila (1982) studied the activity of AchE in different tissues of another cichlid fish, Q. niloticus after she exposed the fish to a sublethal dose of 2.5 mg/l monochrotophos and found that muscle AchE showed the highest inhibition at 98%, while blood AchE was the lowest at 50%, and brain AchE inhibition was about 80-85%. In another study, Sahib and Rao (1980) found that brain AchE activity of the cichlid fish, Q. mossambicus was inhibited by 2.00 mg/l malathion by 47.8% followed by muscle at 45.9% after two days of exposure.

IV. Chlorpyrifos Residues in Fish and Bioconcentration Factor

Many studies have shown that in the natural system, chlorpyrifos concentrations in water decline with sorption on sediments and organic matter (Hulbert et. al., 1970; Schaefer and Duppras, 1970; Mulla et. al., 1973; Roberts et. al., 1973b). Such sorption-desorption processes are considered to be the primary mechanisms for the transient sequestering of chlorpyrifos in the various biotic and abiotic compartments of aquatic ecosystems (Marshall and Roberts, 1978). Studies by Mulla et. al., (1973) indicated that the types of chlorpyrifos formulations and the types of aquatic ecosystem are important factors in the dynamics, distribution, fate, and persistence of chlorpyrifos in the aquatic environment.

Smith et. al., (1966), in their studies of the metabolism of chlorpyrifos C14 labeled chlorpyrifos by goldfish, found that chlorpyrifos was rapidly metabolized and degraded by the fish. They identified five metabolites, the most important of which was 3,5,6-trichloro-2-pyridinol. Thus, in fish, as in mammals and birds, detoxification of chlorpyrifos appears to proceed by hydrolysis to 3,5,6-pyridinol, much of which is then eliminated (Marshall and Roberts, 1978), and this could explain the low concentration of the chemical in fish tissues.

Macek et. al., (1972) found that uptake of chlorpyrifos

by fishes produced maximum residue values of 2.06 mg/kg in bluegills and 2.55 mg/kg in largemouth bass , Micropterus salmoides, within 1-3 days after application of the insecticide at 0.05 lb./A. These residues decreased within two weeks to 0.17 mg/kg in bluegills and were undetectable in the largemouth bass. In another model ecosystem, when goldfish were exposed to 2.5 ug chlorpyrifos/l, residues were detected as 1.6 mg/kg within few hours of exposure (Kenaga, 1972). In a small lake, residues of chlorpyrifos were 0.1 and 0.2 mg/kg in bluegills and largemouth bass respectively, within one week of exposure at a water concentration of 0.25 ug chlorpyrifos/l.

In my study, chlorpyrifos residues in T.zillii adults, after an exposure to 1.7 ug chlorpyrifos/l for 90 days were 0.520 and 0.344 mg/kg in females and males respectively. At 3.6 ug chlorpyrifos/l, residues of chlorpyrifos were significantly higher ($P < 0.05$) in both females and males than at 1.7 ug chlorpyrifos/l (Table 9). However, there was no significant difference between the chlorpyrifos residues of females and males at either concentration.

For the fingerlings exposed to the same chlorpyrifos concentrations for 35 days and fed Purina Trout Chow at 0%, 1%, 2%, 3%, 4%, and 5% of their body weight/day, there was no relationship between the feeding levels and chlorpyrifos residues in tissues (Table 10). This is in contrast to the suggestion of Cripe et. al., (1986) that such relationship between tissue residues and feeding rates should exist.

This finding lends credence to the consideration of Brungs and Mount (1978) who defined the bioconcentration factor as the process by which a compound is absorbed from water through gills or epithelial tissues and is concentrated in the body.

The bioconcentration factor value (BCF) is calculated by dividing the chemical concentration found in the tissues by the measured concentration in the water at equilibrium. The bioconcentration factors of many pesticides and their correlation with physico-chemical properties were well recognized by many investigators. Metcalf et. al., (1974) showed a significant negative correlation between the bioconcentration factor of 49 pesticides in mosquitofish, and the water solubility of pesticides tested. Low water solubility and high lipid solubility can result in BCF values for aquatic organisms of over a million in some cases (Kenaga, 1980).

In other studies, the bioconcentration factors of many chemicals followed a straight-line relationship with the 1-octanol-water partition coefficients (Neely et. al., 1974; Lu and Metcalf, 1975; Neely, 1979; Kanazawa, 1981), and organic carbon soil adsorption (Kenaga and Goring, 1980). An equation of the straight line of best fit for the bioconcentration factors for a variety of chemicals in rainbow trout muscle tissues versus n-octanol-water partition coefficient (P) was developed by Neely et. al., (1974) and used to predict the bioconcentration factors of

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days. Under laboratory conditions, Jarvinen et. al., (1983), using the encapsulated formulation of chlorpyrifos, reported a BCF for fathead minnows of 1673 after an exposure period of 60 days to 0.12-2.68 ug/l using the flow through system. During early life-stage exposures to chlorpyrifos, Cripe et. al., (1986) found a BCF of 118 for sheephead minnows after four weeks of exposure.

In my study, the bioconcentration factors for T.zillii adults exposed for 90 days were estimated to be 202 and 306 for males and females respectively at 1.7 ug chlorpyrifos/l and 500 and 541 at 3.6 ug chlorpyrifos/l (Table 9). When the fingerlings were exposed for 35 days, the bioconcentration factors ranged from 121 to 197 at 1.7 ug/l and 141-250 at 3.6 ug/l (Table 10).

The bioconcentration phenomenon is considered to be one of the most important problems among the chronic effects of pesticides to nontarget organisms, from the viewpoint of protecting the aquatic environment and preventing contamination of the protein resources for mankind (Kanazawa, 1981). Also, by knowing the potential of new materials, including pesticides, and by matching of this potential with the intended end use, an early judgment decision can be made as to the possible long-term environmental problems that may be faced (Neely et. al., 1974).

V. Ecosystem Impact

It has been estimated that less than 0.1% of the insecticides applied for agricultural purposes actually reaches the target pests (Pimentel and Levitan, 1986). The remaining could reach and contaminate land, air, and water and adversely affect nontarget organisms. Insecticides have long been used in or near aquatic ecosystems with little concern for factors other than the efficiency of the insecticides against target organisms (Johnson and Mayer, 1972).

Water pollution by insecticides became a problem in the 1940's concurrently with the rapid advances in pest control made possible by the development of new synthetic insecticides where many of which are remarkably lethal to aquatic forms of life (Nicholson, 1969). Both short and long term contamination of the environment by different kinds of insecticides have become much more difficult to evaluate.

The original problems were caused primarily by the organochlorine insecticides (e.g., DDT), because of their low water solubility and high persistence in the environment. This concern was supported by evidence that some of these organochlorine insecticides can accumulate in subsequently higher steps in the food chain. The mechanism by which this is achieved is called biological magnification (Thoman and Nicholson, 1963). The disastrous implications of biological magnification were recognized and

discussed in Rachel Carson's 1962 book, "Silent Spring", which led to a full-scale public debate about the environmental hazards of pesticides.

In the 1950's and 1960's, the less persistent organophosphorus insecticides, began to replace the highly persistent or "hard" organochlorine insecticides. In many instances these compounds enter the aquatic environment not only by chance runoff from application to agricultural lands, but also by direct application to aquatic ecosystems for insect control purposes (Weiss, 1961). Although many of the organophosphorus insecticides are generally less toxic to fish, they are much more toxic to mammals than the organochlorine insecticides (Henderson and Pickering, 1957), and highly toxic to fish food organisms (Holden, 1964). During the past two decades, the need for information on the chronic exposure on the sublethal effects of many organophosphorus insecticides on aquatic organisms and ecosystems has become evident (Duke et. al., 1977).

In my study, the fish T.zillii, was exposed to the organophosphorus insecticide chlorpyrifos in acute and chronic studies to evaluate its various adverse effects on the fish. From the present investigation, I calculated the acute 96-h LC50 value for chlorpyrifos to be 240 ug/l. In the chronic studies, spawning and reproductive behavior, survival of early life stages, and growth of T.zillii were impacted at a concentration as low as 3.6 ug chlorpyrifos/l, which is almost two orders of magnitude smaller than the

acute value. Accordingly, the maximum acceptable toxicant concentration (MATC) or the "no effect" level of using chlorpyrifos was found to be between 1.7 and 3.6 ug/l and the application factor to be in the range of 0.007-0.015 (Table 12). In addition, the chronic value was calculated to be 2.474 ug/l.

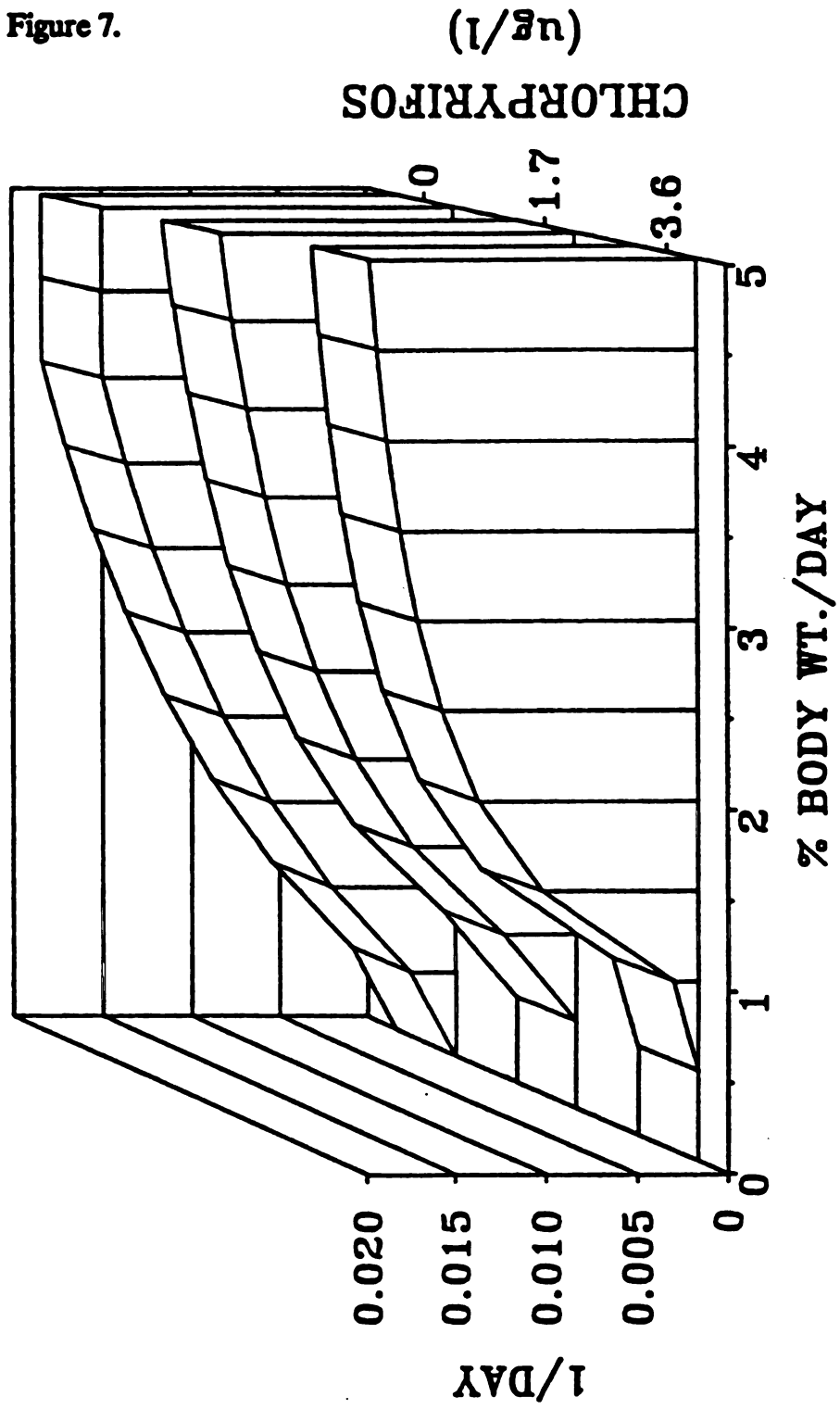
It appeared from these studies that the impact of chlorpyrifos on the growth of T.zillii was the most sensitive parameter among the various measures considered. In spite of this impact of chlorpyrifos on the growth rate, reductions in food availability caused bigger reductions in the growth rate of T.zillii than did the increase in chlorpyrifos concentration (Figure 7).

The larvae of most fish, including the tilapia species, feed initially on aquatic invertebrate populations, especially zooplankton organisms (Torrans, 1986). However, many aquatic invertebrates are reported to be much more sensitive to insecticides, and in these cases, the safe and acceptable levels for fish production may not be adequate to protect the fish-food species (Mount, 1967). Reduction in the food for the young fish would reduce their growth and survival, thereby reducing recruitment of young fish to wild populations.

Many of the aquatic zooplanktonic species have been reported to be very sensitive to chlorpyrifos (Sanders, 1969; Johnson and Finley, 1980; Siefert et. al., 1988). The calculated LC50's for different zooplankton species with

Figure 7. The three-dimensional relationship between the specific growth rate response and both food ration and chlorpyrifos concentration for T.zillii.

Figure 7.

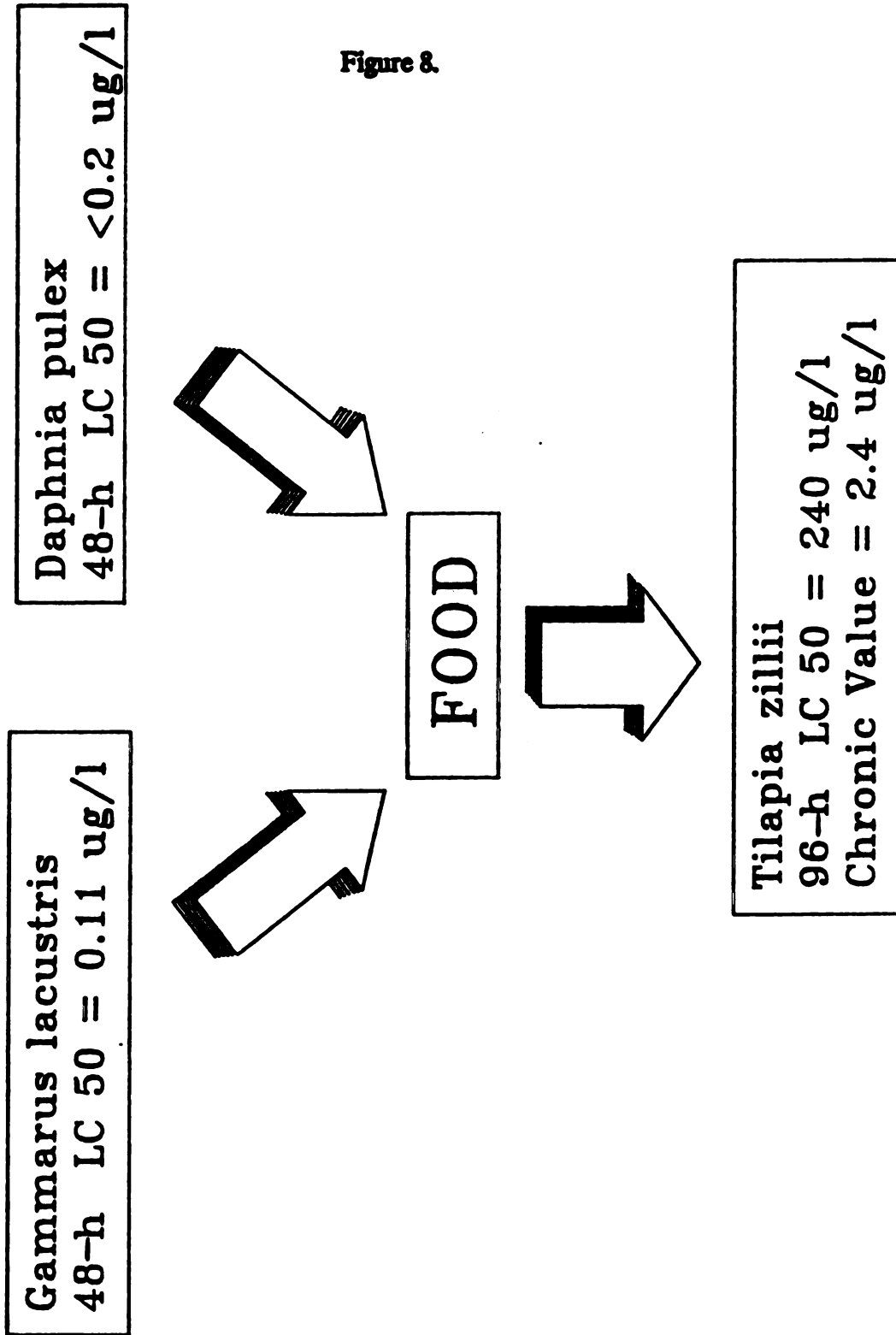


chlorpyrifos were reported to be as low as 0.11 ug/l (96-h LC50) for the amphipod, Gammarus lactustris (Sanders, 1969) and < 0.5 ug/l (48-h LC50) for another amphipod, Hylella azeta and < 0.2 ug/l for Daphnia pulex (Siefert et. al., 1988). Also, the order of susceptibility of aquatic insects to chlorpyrifos is mosquito larvae > dragonfly naiads = damselfly naiads = stoneflies > caddisflies > corixids (Marshall and Roberts, 1978). If the impact of chlorpyrifos on these organisms follows the pattern I noted for T.zillii, the chronic effect concentrations must be extremely minute.

In addition, chlorpyrifos was found to reduce, change, or eliminate the populations of many aquatic invertebrates. The caddisfly populations were eliminated in ponds treated with chlorpyrifos at 0.05 lb./A (Macek et. al., 1972). In the same ponds, mayfly populations were reduced to less than 10% of those in control ponds, and the relative numbers of midges emerging were significantly reduced by approximately 65%. In another study, the amphipod communities were significantly changed to isopods in outdoor experimental streams subjected to a continuous exposure of chlorpyrifos at an average of 0.35 ug/l (Eaton et. al., 1985).

Thus, the estimated safe level, or the "no effect" value, of 2.474 ug chlorpyrifos/l that I calculated from my studies for T.zillii, would appear to reduce or eliminate the amphipods, cladocerans, and other zooplankton populations critical for the continued survival of the young fish (Figure 8). The reduction or elimination of these

Figure 8. Toxicity of chlorpyrifos to the food organisms for young T.zillii compared with the toxicity of chlorpyrifos to T.zillii.



aquatic zooplankton populations, which are the main source of food for early life stages of the fish, would indirectly affect the growth and survival of fish, and gradually reduce or eliminate the fish population in a pond, lake, or stream, through reductions of recruitment of young fish to the adult population.

The numerical water quality criteria for chlorpyrifos by the United States Environmental Protection Agency (1986) to protect freshwater aquatic life are 0.041 ug/l (four-day average concentration) and 0.083 ug/l (one-h average concentration) either of which should not be exceeded more than once every three years on the average. Although the three years is the Agency's best scientific judgment of the average time for aquatic ecosystems to recover, resiliences of ecosystems and their abilities to recover differ greatly (United States Environmental Protection Agency, 1986).

Because of such possible adverse effects they may have on nontarget aquatic organisms, the introduction and use of agricultural insecticides must be controlled in some manner to reduce risks associated with their use to a minimum. Most developed countries exercise such control through some legislation regarding the use of insecticides in agriculture. The problem of water contamination by insecticides used in agriculture is much more serious in developing countries where the control on their use is minimal. Yorinori (1983) noted the following shortcomings associated with pesticide usage in many developing

countries:

"a. At the government level, the various agencies acting in the area of pesticides are not properly integrated, and often there is overlapping of activities.

b. The industry and pesticide dealers are primarily interested in their profit and price control by the government is often lacking.

c. The personnel involved in the inspection of pesticide products and sanitary education is minimal when compared with the complexity and extent of the problem.

d. The farmer is often led to use a pesticide before he knows how to handle it, and all the information contained on the label is useless when he cannot read or does not care about it.

e. The enforcement of the existing laws is sometimes very poor.

f. Finally, the consumer is the victim of the consequences of improper use of pesticides, without any option."

In summary, to ensure continuity of aquatic ecosystems, factors such as interrelationships of fish, fish-food organisms, and plants must be considered when these systems are likely to become directly or indirectly contaminated with insecticides used on croplands (Johnson and Mayer, 1972). Also, protection of aquatic ecosystems by regulating agricultural pesticides with consideration of only acute and chronic toxicity tests on fish is not enough. In fact, no amount of research can eliminate all the uncertainties and

implications associated with assessing the risks from using the hazardous agricultural chemicals in or nearby aquatic ecosystems (Young, 1987).

However, a number of alternatives to the use of insecticides in agriculture could serve to minimize their adverse impact on the environment and aquatic ecosystems. One of the most promising alternatives is the Integrated Pest Management (IPM). In this method, the physical, cultural, and biological pest management techniques are integrated with the use of the safest possible chemical pesticides, where necessary, into a strategy which could deal with agricultural pest problem as a part of a system, including people, crops, beneficial insects, fish and wildlife, as well as pests and chemicals (Bull, 1982).

In the final analysis, education of the public and pesticide users concerning the different consequences and implications of pesticide misuse is probably the most important step. Minimizing the hazard from agricultural chemicals also calls for improved laws and enforcement of effective regulations for licensing pesticide applicators and precisely labeling each pesticide product. These various attempts to control the adverse impact of agricultural insecticides on the aquatic ecosystems will be extremely difficult to invoke, especially in developing countries.

CONCLUSIONS

1. Chlorpyrifos is highly toxic to T.zillii and survival of early life stages, reproductive behavior and brain AchE activity were significantly impacted at a concentration as small as 3.6 ug chlorpyrifos/l, which is almost two orders of magnitudes smaller than the 96-h LC50 of 240 ug/l.
2. The specific growth rate of the fish, T.zillii, was the most sensitive parameter measured and was affected at chlorpyrifos concentration as low as 1.7 ug/l.
3. A chlorpyrifos concentration of 3.6 ug/l caused about 80% inhibition of AchE in females, which was significantly higher than in males (73%), may explain the failure of T.zillii to spawn.
4. The maximum acceptable toxicant concentration (MATC), or "no effect" level, for chlorpyrifos and T.zillii, was estimated to be between 1.7 and 3.6 ug/l and the application factor (AF) was in the range of 0.007-0.015, and the chronic value was 2.474 ug/l.
5. Chlorpyrifos tends to accumulate and bioconcentrate in T.zillii muscle tissues, and there was neither significant difference between adult males and females residue content nor significant effect of feeding levels on residues in fingerlings.

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