

CHRONIC TOXIC EFFECTS OF THE ORGANOPHOSPHORUS INSECTICIDE
CHLORPYRIFOS (DURSBAN) ON THE SPAWNING, REPRODUCTIVE BEHAVIOR AND
EARLY LIFE STAGES OF THE CICHLID FISH TILAPIA

MOHAMED M. SHEREIF

DEPARTMENT OF PLANT PROTECTION, FACULTY OF AGRICULTURE,
UNIVERSITY OF AIN SHAMS, EGYPT

Abstract: The fish Tilapia zillii was exposed to sublethal concentrations of the organophosphorus insecticide chlorpyrifos (Dursban) using the continuous flow-through system to evaluate its chronic toxic effects on spawning, reproductive behavior, and early life stages of the fish. Chlorpyrifos affected spawning and reproductive behavior at concentrations as small as 3.6 ug/l. Also, survival of early life stages of T. zillii was reduced significantly at the concentration of 3.6 ug/l than at 1.7 ug/l or the control. The maximum acceptable toxicant concentration (MATC), or "no effect" level was estimated to be between 1.7 and 3.6 ug/l and the application factor (AF) was in the range of 0.007 and 0.015, and the chronic value was 2.474 ug/l.

INTRODUCTION

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. However, the adverse impact of pollutants, including all pesticide groups, is much greater on the aquatic

organisms than on the terrestrial organisms [1].

Organophosphate pesticides have been viewed as being less hazardous to biota than chlorinated ones because they are less persistent in the environment. However, some of the organophosphorus pesticides are as acutely toxic as organochlorine pesticides [2].

In this study, the cichlid fish, Tilapia zillii, was exposed to the organophosphorus insecticide chlorpyrifos (Dursban) during chronic laboratory studies conducted under continuous flow conditions. The objectives of this study were to evaluate the chronic toxic effects of chlorpyrifos on spawning, reproductive behavior and survival of early life stages of the fish; and to use such chronic data to determine the "safe" level of using chlorpyrifos, the chronic value, and the application factor (AF) for chlorpyrifos on T.zillii.

MATERIAL AND METHODS

All chronic experiments in this study were carried out at the Department of Fisheries and Wildlife and Pesticide Research Center, Michigan State University, U.S.A., using a proportional diluter [3,4] of the solenoid valve type (Ace Glass Inc., Vinland, N.J.). This diluter allowed maintenance of constant concentrations of chlorpyrifos in the exposure tanks. Two sublethal concentrations of chlorpyrifos were chosen (1.7 and 3.6 ug/l) for conducting all chronic experiments. Chlorpyrifos concentrations were monitored weekly by reverse-phase High Performance Liquid Chromatography (HPLC). Dissolved oxygen, pH, and total alkalinity and hardness of water in all exposure tanks

were monitored weekly, while tanks water temperature was monitored daily (Table 1).

I. Spawning and reproductive behavior

Healthy mature males and ripe females were transferred from the adult brood stock into duplicates of 70 gallon aquaria (approximately 120 liters) and exposed to two sublethal concentrations of chlorpyrifos (1.7 and 3.6 ug/l) for about 90 days. Sexing of fish was based on the structure and form of the genital papillae [5]. 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 clean sand to enhance nest building by fish. Fish were fed daily to satiation with purina trout chow food. 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 were removed after the two weeks parental care period. All tanks were cleaned thoroughly between spawns.

II. Early life stages:

The newly hatched larvae of T. zillii (< 24 hr.) were collected from brood tanks and exposed in duplicates to the same two sublethal concentrations of chlorpyrifos (1.7 and 3.6 ug/l) for 28 days. Fifty hatched larvae were put in each of the five gallon tanks (filled to contain 8.5 liters). After the larvae exhausted their yolk sacs, they were fed very fine powdered purina trout chow to satiation, and any food left was carefully siphoned out of the exposure tanks.

One-way analysis of variance (ANOVA) was used to evaluate

differences in chronic effects of chlorpyrifos on both the spawning and reproductive behavior and early life stage survival of T. zillii. Dunnet's test [6] was then used to compare the treatment means with control means.

RESULTS

I. Spawning and reproductive behavior:

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 by 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 completely 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 was 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 2).

Table 1. 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 $\pm$ 1

Table 2. Effect of chlorpyrifos on spawning and reproductive behavior of *T.zillii* exposed to 1.7 and 3.6 ug/l for 90 days through two spawns.

Measured conc. (ug/l)	Developmental stage (days)			
	Hatching period	Yolk sac adsorption	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

Table 3. Survival of early life stages of *T.zillii* exposed to 1.7 and 3.6 ug chlorpyrifos/l for 28 days.

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

II. Early life stages:

As shown in Table (3), all 50 larvae fish survived in the control, one mortality at 1.7 ug chlorpyrifos/l, and 15 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 or the control.

DISCUSSION

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/or growth of the fish [1]. Therefore, chronic toxicity tests are now commonly conducted to provide a more sensitive measure of chemical toxicity than is allowed by acute toxicity tests [7]. 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 some of the adverse toxic effects of the chemical(s) under study.

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 [8,9]. 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 (NOEC) [10]. In addition, when the maximum acceptable toxicant concentration (MATC) is divided by the concentration causing 50% mortality (LC50), the result is the

الطبخ العربي

تم في هذه الدراسة تعريف أسماك البلطي الأحمر تركيزات تحت مينة من المبيد الفوسفوري الكلوربيرفوس (الدروسيان) تحت نظام التيار المستمر من المبيد وذلك باستخدام جهاز "الديليوتر" بغرض تقييم تأثير السم المزمّن على عدد مرات وسلوك التنفس وكذلك حياة الأعمار الصغيرة من هذه الأسماك . وقد وجد أن التركيز ٣ ميكروجرام / لتر من مبيد الدروسيان قد أثر على عدد مرات وسلوك تنفس هذه الأسماك كما تأثرت حياة الأعمار الصغيرة من الأسماك عند هذا التركيز . غذا تم استنتاج بعض قيم تأثير السم المزمّن لهذا المبيد لأسماك البلطي الأحمر مش حدود الأمان لاستخدام المبيد ومعايير التسميم وكذلك القيمة المزمّنة والتي يمكن السطاح بها الحماية من هذه الأسماك في بيئتها المائية .