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Effects on Reproduction in the Guppy (*Poecilia reticulata*) Under Chronic Exposure to Temephos and Fenitrothion

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Both temephos and fenitrothion are well known as relatively safe insecticides since their mammalian toxicity is low. The former is used for the control of blackfly, mosquito and midge at the aquatic stages and, therefore, fish are often exposed to this chemical. The latter insecticide is used most commonly for pest control. Much information has accumulated on the toxicity of both insecticides to fish (SASA et al. 1965, NISHIUCHI and HASHIMOTO 1968, VERMA et al. 1978, TSAI 1978). Most of the studies have been concerned with acute toxicity but the effect of sublethal concentrations of these insecticides to fish over a long exposure period have not been sufficiently studied. CARDWELL et al (1977) reported that technical chlordane inhibited egg production in bluegills and brook trouts during chronic exposure. On the other hand, lindane did not affect the reproduction in fathead minnows (MACEK et al 1976). As for organophosphorous insecticide, EATON (1970) reported the chronic toxicity of malathion to the bluegill but did not find a significant effect on reproduction. The focus of the present investigation was, therefore, placed on examining the effects of sublethal levels of temephos and fenitrothion on reproduction in the guppy, *Poecilia reticulata*.

MATERIALS AND METHODS

Poecilia reticulata used in the present study was the strain selected and colonized at the National Institute for Environmental Studies, Japan. They were reared for three months after birth in a aqualium in which the light and temperature were controlled at 3000 lux for a 14 hour light and 10 hour dark photoperiod and 23±1° C respectively. Thereafter ten females and three males were transferred as a group into a glass exposure tank of 60 l capacity (30 cm x 60 cm x 35 cm) containing 40 l water. The weight of fish at this time ranged from 0.57 g - 0.63 g for the females and 0.23 g - 0.27 g for the males. A four month pre-exposure period had been performed in order for fish to aclimatize to the experimental conditions. During this period as well as during the exposure and post-exposure periods, the adult fish were put into a breeding cage made of nylon netting (17 cm x 50 cm x 17 cm with 3 mm apertures) suspended in the exposure tank, so that predation by adults on fry was prevented. Fry escaped from the cage through the mesh immediately after birth and, therefore, a daily record of the exact number of them could be made. Fish were fed dry trout once a day. The insecticides were applied as emulsions for the various concentration levels. The concentrations of insecticide tested were 0.25 ppm, 0.5 ppm, 1.0 ppm and 1.5 ppm for temephos and 0.1 ppm, 0.25 ppm, 1.0 ppm and 1.5 ppm for fenitrothion in 40 l of water under semi-static conditions. Half of the water volume was renewed daily with fresh water containing appropriate concentrations of insecticide. Dilution was made with underground water which was filtered and aerated prior to use. The exposure to the chemicals was continued for a period of two months and observation was extended for a further two months. Young fry produced as well as residues of food were removed daily before renewal of water. In the experiment with temephos, youngs were reared for two weeks to observe their survival rate and thereafter weighed individually. In the case of abnormal birth, the development stage of the embryo was recorded. In the experiment with fenitrothion, 10 individuals were selected at random and were weighed weekly soon after parturition. Since the brood size in the guppy shows a cyclic variation (ROSENTHAL 1952) and also the cycle of parturition by females

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in each breeding cage did not necessarily proceed simultaneously, the number of youngs produced was summed monthly.

RESULTS

1) Experiment with temephos

During the course of this experiment, no fish died in the control but three females and two males died at a concentration of 1.5 ppm one day after the beginning of the exposure and one more female died at the same concentration 13 days after. For the other concentrations, only one female died respectively. In these cases fish were not replaced. Thus, the number of youngs produced per chamber was converted to that per female per month for comparison.

TABLE 1

Normal and premature parturition by the guppy before, during and after exposure to various concentrations of temephos

Concentration (ppm)		Pre-exposure		Exposure		Post-exposure	
		1st month	2nd month	1st month	2nd month	1st month	2nd month
Control	Live fry	6.4	16.6	6.8	11.0	6.3	6.2
	Dead fry	0	0.3	0.2	0.3	0.4	0.3
	Eyed embryo	0	0	0	0	0	0.4
	Undeveloped embryo	0	0	0	0	0.1	0
	Total	6.4	16.9	7.0	11.3	6.8	6.9
0.25	Live fry	16.2	8.8	8.4	6.6	4.4	2.9
	Dead fry	0.2	0.8	0.8	1.3	0.1	0.6
	Eyed embryo	0	0	0	1.3	0.9	0.1
	Undeveloped embryo	0	0	0	3.6	0.7	1.4
	Total	16.4	9.6	9.2	12.8	6.1	5.0
0.5	Live fry	7.1	6.8	20.0	2.1	5.0	4.4
	Dead fry	0.1	0	1.0	1.1	0.2	2.1
	Eyed embryo	0	0	0	0.2	1.9	1.1
	Undeveloped embryo	0	0	0	0	0	1.0
	Total	7.2	6.8	21.0	3.4	7.1	8.6
1.0	Live fry	18.8	9.1	6.6	3.0	3.0	1.9
	Dead fry	0	0	1.2	1.2	0.1	0.1
	Eyed embryo	0	0	0	1.6	3.8	1.7
	Undeveloped embryo	0	0	0	0	0	7.2
	Total	18.8	9.1	7.8	5.8	6.9	10.9
1.5	Live fry	9.5	8.7	3.4	1.2	0.2	0
	Dead fry	0	0	0.9	1.3	0.8	0.7
	Eyed embryo	0	0	0.5	0	2.3	1.2
	Undeveloped embryo	0	0	0	0	1.8	13.8
	Total	9.5	8.7	4.8	2.5	5.1	15.7

Numerals indicate numbers produced per female per month.

As far as the total number of eggs produced, including live fry as well as dead embryos, it was found that there was no significant difference between months and also between concentrations (Table 1). However, the number of live juveniles produced at 1.5 ppm clearly decreased one month after the exposure and the effect continued at least two months and possibly more after termination of exposure. It must be stated that a clear effect was not noted during the first month of the exposure. We may recognize a slight increase of still birth for every concentration level during the exposure period. During the post-exposure period, premature parturition occurred among females exposed to the chemical. The number of abnormal parturitions seems to be correlated with the concentration of temephos. The premature births at 1.0 ppm and 1.5 ppm during the first month of the post-exposure included a considerable number of eyed period eggs and for the second month included much more undeveloped eggs.

The survival rates of young fry reared for two weeks after birth in this experiment did not differ significantly for the various concentrations. Some youngs died during the first week in every concentration level including the control but few fish died during the second week.

TABLE 2

Normal and premature parturition by the guppy before, during and after exposure to various concentrations of fenitrothion

Concentration (ppm)		Pre-exposure		Exposure		Post-exposure	
		1st month	2nd month	1st month	2nd month	1st month	2nd month
Control	Live fry	12.7	18.7	14.2	7.8	6.1	4.5
	Dead fry	0.6	0.1	0.2	0.6	0.3	0
	Eyed embryo	0.2	0.2	0.1	0.6	0.5	0
	Undeveloped embryo	0	0	0	0.1	0.4	0
	Total	13.5	19.0	14.5	9.1	7.3	4.5
0.1	Live fry	13.5	19.6	11.5	5.8	2.3	6.8
	Dead fry	0.7	0.2	0.5	0.4	0.6	0.8
	Eyed embryo	0.2	0.5	0.2	0	0.2	0.1
	Undeveloped embryo	0.6	0.2	0.3	0	0	0.6
	Total	15.0	20.5	12.5	6.2	3.1	8.3
0.25	Live fry	15.2	16.9	9.5	1.3	1.4	2.6
	Dead fry	0.8	0.5	1.0	0.1	0.3	0.5
	Eyed embryo	0.1	0.3	0.7	0	0.2	1.4
	Undeveloped embryo	0	0.1	1.8	0.1	3.6	3.0
	Total	16.1	17.8	13.0	1.5	5.5	7.5
0.5	Live fry	17.9	15.1	10.9	1.5	0.9	2.0
	Dead fry	1.2	2.1	1.9	0.5	0	2.1
	Eyed embryo	0	0.2	0.4	0	0.1	1.3
	Undeveloped embryo	0	0.1	0.4	0	0.6	4.4
	Total	19.1	17.5	13.6	2.0	1.6	9.8
1.0	Live fry	15.3	14.3	10.2	0.3	0	0
	Dead fry	0.5	0.3	1.9	0.3	0	0
	Eyed embryo	0.1	0.3	0.7	0	0	0
	Undeveloped embryo	0	0.1	0.5	0	0.3	8.6
	Total	15.9	15.0	13.3	0.6	0.3	8.6

Numerals indicate numbers produced per female per month.

2) Experiment with fenitrothion

During this experiment, the mortality of females was least; one female exposed to 1.0 ppm fenitrothion died nearly at the end of the two month exposure period. In this experiment also, the overall fecundity was not affected during the first month of the exposure; namely, females exposed to the chemical produced almost the same number of live fry as in the control (Table 2). During the second month of the exposure, the number of young produced decreased significantly at 0.25 ppm, 0.5 ppm and 1.0 ppm. The insecticide seems to inhibit egg production itself. After termination of exposure, the rate of the egg production tended to recover but the effect remained at a concentration of 0.5 ppm and 1.0 ppm during the first month of the post-exposure period. The effect of this chemical at 1.0 ppm on the reproduction of the guppy was so serious that all females aborted at an early stage of the embryo even during the second month of the post-exposure. A significantly high rate of abortion was observed also at 0.25 ppm and 0.5 ppm during the same period.

The weight of fry at birth before and after exposure did not differ significantly up to 0.25 ppm compared with the control but decreased at 0.5 ppm in the post-exposure months (3.75 ± 0.76 - 3.86 ± 0.97 vis 4.49 ± 0.35 - 4.87 ± 0.68 in control).

DISCUSSION

Organophosphorous insecticides are easily metabolized in the body of fish and are excreted rapidly. Fenitrothion is not exceptional. KANAZAWA (1975) and TAKIMOTO and MIYAMOTO (1976) reported a rapid excretion of this chemical from motsugo or rainbow trout. Therefore, the insecticide seemed not to remain long in the body after the discontinuation of exposure. Nevertheless, both temephos and fenitrothion affected the reproduction in the guppy after termination of exposure. The cholinesterase inhibition remains for a while after exposure in many cases of organophosphorous insecticides (MACEK et al. 1972, POST and LEASURE 1974). However, it is not known if the retaining of the reduction in the activity of cholinesterase is related to an impairment of the reproduction. KAPUR et al. (1978) suggested fenitrothion affected reproduction of fish after studies on the activity of enzymes indicated that steroidogenesis in the gonad of *Cyprinus caprio* was related to the exposure of the insecticide.

In the present study, temephos reduced the normal birth and fenitrothion reduced the egg production during the exposure. It must be noted that such effects do not appear during the first month, suggesting that these chemicals do not stop the development of advanced oogenesis. It is of interest that the effect of temephos is more significant during the later period of post-exposure. As for fenitrothion, the effect on the reproduction tended to recover after discontinuance of exposure except at 1.0 ppm where females aborted all eggs before development. BOYD (1964) reported a case of abortion in the mosquito fish for exposure to DDT. This must be a characteristic phenomenon found in viviparous fish. Zinc also impaired the reproduction in the guppy (UVIOVO and BEATTY 1979) by reducing the egg production and also by reducing the body size of the youngs. In the present study, the survival of youngs from normal birth did not differ between the exposed and control but a slight difference was found in the weight among youngs at birth.

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