AN ANALYSIS OF THE SWIMMING BEHAVIOR OF FISH EXPOSED TO THE INSECT GROWTH REGULATORS, METHOPRENE AND DIFLUBENZURON

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ABSTRACT. Locomotor activities of mosquitofish (*Gambusia affinis*) and goldfish (*Carassius auratus*) were monitored for a 2-week period in the presence of insect growth regulators, at concentrations approximately 10-fold greater than those generally recommended for application. Methoprene, the active ingredient in Altosid® SR-10, at 0.2 ppm did not significantly alter the locomotor activity of either mosquitofish or goldfish. Diflubenzuron (trade name, Dimilin®) at 0.2 ppm caused a temporary hyperactivity in mosquitofish. Within 2 days following exposure the fish became about 2.5 times more active than controls. Maximum activity was observed on days 4 through 8 when they were 4 times as active as controls. Activity then decreased to control levels by day 14, suggesting that the mosquitofish were able to adjust to or compensate for the presence of diflubenzuron.

Mosquito control agencies are faced with the problem of controlling mosquito populations by methods which will not harm other members of the ecosystem. Hence the interest in the use of recently developed larvicides or insect growth regulators (IGR's).

At the present time methoprene (isopropyl (2E, 4E)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate), and diflubenzuron (N-(4-chlorophenyl)-N′-(2, 6-difluorobenzoyl) urea) are generally considered to have the greatest potential for immediate use. They are especially attractive in that they appear to be somewhat specific for mosquitoes and are rapidly biodegraded, with half-lives of 1 week or less. Methoprene, a juvenile hormone analog, inhibits adult emergence from the pupal case (Schaefer and Wilder 1972), whereas diflubenzuron appears to exert its lethal effect by acting as an inhibitor of chitin synthesis (Clarke et al. 1977).

While toxicity tests have been carried out on the aforementioned IGR's to arrive at recommended doses which result in no mortality in non-target organisms, there have been fewer studies to determine whether sub-lethal effects in non-target organisms occur at recommended usage levels. Studies to examine this possibility are of importance in that sub-lethal effects on any member of an ecosystem might seriously affect the delicate balance of the entire ecosystem (Barber et al. 1978).

In the present investigation the effects of methoprene on the locomotor activities of mosquitofish (*Gambusia affinis*) and goldfish (*Carassius auratus*) and the effects of diflubenzuron on the locomotor activities of mosquitofish have been examined using the methods of Ellgaard et al. (1975) which result in a mathematical description of activity. Locomotor activity was chosen for these studies since it is a suitable indicator of the general health of fish (Malizia et al. 1975).

MATERIALS AND METHODS

Mosquitofish, *Gambusia affinis,* were obtained from the Jefferson Parish Mosquito Control Department and goldfish, *Carassius auratus,* were purchased from a local fish store. In the laboratory they were transferred to dechlorinated tap water at 22 ± 1°C and pH 6.5 under continuous light. Aquarium water was air-saturated using air stones and external filters which contained glass wool and activated charcoal. Fish were maintained in continuous light and fed daily, but never within 60 min of experimental periods. Experiments were carried out at the same time each day.

Experiments were run in 38 liter

aquaria (51 × 32 × 27 cm). Prior to an experimental run all aerators and filters were removed. Each run consisted of 30 fish, either mosquitofish (4.0 cm average total length) or goldfish (6.0 cm average total length). At the beginning of a run the fish were gently moved to one half of the aquarium (compartment A) by means of a perforated screen. A Plexiglas partition containing 4 evenly-spaced holes (4.0 cm diam.) was then inserted alongside the screen. After the fish had voluntarily distributed themselves in a random fashion in compartment A (5 min), the screen was removed allowing the fish to move through the Plexiglas partition into the other half of the aquarium (compartment B) and thus distribute themselves throughout the entire aquarium. The number of fish found in compartment B was determined at 1-min intervals following removal of the perforated screen. The fish movements under these conditions describe an opposed first-order reaction which can be expressed in terms of its rate by the following equation:

$$Kt = (Ka + Kb)t$$
$$= \ln [((A)_o - (A)_e) / ((A) - (A)_e)],$$

where ln = natural logarithm, (A) = number (concentration) of fish in compartment A at time t, $(A)_0$ = number (concentration) of fish in compartment A at time t = O, $(A)_e$ = number (concentration) of fish in compartment A at equilibrium, K = sum of the rate constants for the forward and reverse reactions, or $K_a + K_b$, and t = time (in these studies it is time after removal of the perforated screen to allow fish to begin dispersing).

In an opposed first-order reaction when $\ln [((A)_o - (A)_e) / ((A) - (A)_e)]$ is plotted against t, a straight line is obtained. The slope of such a line is equal to the rate constant, K (reaction rate) of the reaction.

To determine the effects of the larvicides on motility, daily rate constants were determined on control and treated groups of fish. Mosquito-fish were exposed to methoprene (initial concentration, 0.2 ppm) for 12 days or diflubenzuron (initial concentration, 0.2 ppm) for 15 days. Goldfish were exposed to methoprene (initial concentration, 0.2 ppm) for 13 days. Control groups were maintained in tap water and examined at the same times as treated fish. Kinetic experiments were conducted once daily on all groups. In an attempt to counterbalance its decay and maintain constant exposure levels, methoprene was added every 2 days in an amount so as to increase the concentration remaining after each 2-day interval by 0.1 ppm. Similarly, diflubenzuron was added on day 8 in an amount to increase its concentration by 0.1 ppm over that remaining after 8 days of degradation.

The mortality of fish in each group was recorded during the course of the experiments. Dead fish were replaced with fish from holding tanks containing either tap water (controls) or larvicide at the appropriate concentration.

RESULTS

A rate constant was determined each day for each group of fish from a log-rate plot as typified by Fig. 1.

The average rate constants (± standard deviation) for the locomotor activities of control and methoprene-treated mosquitofish for the 12-day experimental period were 0.502 (±0.434) and 0.270 (±0.377) respectively. The average locomotor rate constants (± standard deviation) of control and methoprene-treated goldfish for the 13-day experimental period were 0.133 (±0.069) and 0.164 (± 0.088) respectively. In both sets of experiments there were no significant differences, as judged by the Student's t-test, in the locomotor rates of control and methoprene-treated fish. The results are presented as average rate constants for the entire treatment period since variations in daily rate constants did not indicate any significant temporal trend during the exposure period.

Rate constants for locomotor activity of

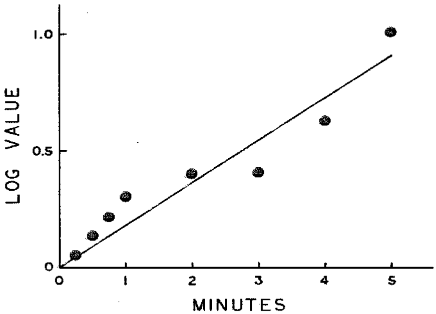

Fig. 1. Typical reaction kinetics for locomotor activity of goldfish. The value of ln $[((A)_0 - (A)_e) / ((A) - (A)_e)]$ is plotted against time, t. The time in such a reaction is minutes (or fractions thereof) after removal of the perforated screen to allow the fish to begin dispersing. The slope of the best-fit line, as determined by the least-squares method, is equal to the rate constant, K, for this experiment.

mosquitofish exposed to diflubenzuron varied with time of exposure as shown in Figure 2. A repeat of this experiment demonstrated similar results (not shown) with maximal activity again observed on day 6 of exposure to diflubenzuron and return to control level activity by day 14.

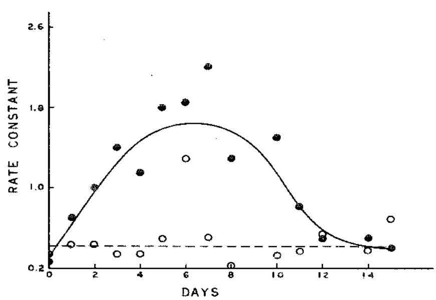

Fig. 2. Rate constants for locomotor activity at various times after exposure of mosquitofish to diflubenzuron. On day 0 the aquaria were made 0.2 ppm with respect to diflubenzuron and on day 8 diflubenzuron was added to increase the remaining concentration by 0.1 ppm.

In all experiments there was no significant difference in mortality for control or treated fish, with both groups showing an average rate of 1 death every 2 days.

DISCUSSION

It previously has been demonstrated that potential sub-lethal effects of various agents on fish can be detected by examining their swimming activities. Ellgaard et al. (1977) have shown that sub-lethal concentrations of DDT effect changes in locomotor activity of bluegill sunfish, *Lepomis macrochirus*, in a concentration-dependent fashion. In fact at DDT concentrations as low as 0.008 ppb (greater than a 25-fold dilution of the 96-hr mean lethal concentration, or 96 hr LC_{50}) such fish are 1.3 times as active as controls. Locomotor hyperactivity is also observed to be concentration-dependent in bluegill exposed to sub-lethal concentrations of cadmium, chromium or zinc (Ellgaard et al. 1978).

The present study demonstrates that methoprene at a concentration of 0.2 ppm, which is 10-fold greater than that recommended for field application, does not significantly affect the locomotor activities of mosquitofish or goldfish. Since little mortality was observed during these studies and since Miura and Takahashi (1973) have observed that *Gambusia* survive concentrations greater than 8.0 ppm of methoprene, 0.2 ppm methoprene may be considered a sub-lethal concentration which does not elicit a measurable sub-lethal locomotor response by either mosquitofish or goldfish over the course of a 2-week exposure period. Whether effects might become apparent after longer periods following exposure, as has been observed in other animal populations by Breaud et al. (1977), remains to be determined. However, Takahashi and Miura (1975) found that the growth of populations of *G. affinis* in ponds treated monthly (over 5 months) with Altosid (0.03 lb active ingredient/acre) was not affected.

In contrast to methoprene, diflubenzuron at a concentration of 0.2 ppm effected hyperactivity in mosquitofish. Within 2 days following exposure, fish became about 2.5 times as active as controls. Maximal activity was observed on days 4 through 8, during which time the treated fish were about 4.0 times as active as controls. Then activity began to decrease and returned to control levels of locomotor activity by day 14. Since diflubenzuron was added for a second time on day 8, the return to normal locomotor activity indicates that the mosquitofish are able to adjust to or compensate for the presence of diflubenzuron. Thus, this effect of diflubenzuron on the mosquitofish would appear to be transient. Since little mortality was observed at the 0.2 ppm concentration used here, these effects may also be viewed as sub-lethal. In fact, Miura et al. (1975) have observed that mosquitofish survive in concentrations of diflubenzuron as great as 80 ppm and Julin and Sanders (1978) have determined that the LC_{50} of diflubenzuron exceeds 100 ppm for 4 other species of fish.

Although the mechanism by which diflubenzuron elicits this temporary hyperactivity is not explored in the present studies, it has been suggested that changes in locomotor activity of fish reflect alterations in their basic metabolism (Malizia et al. 1975).

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