SUBLETHAL EFFECTS OF LARVAL METHOPRENE EXPOSURE ON ADULT MOSQUITO LONGEVITY

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ABSTRACT. Larvae of *Aedes aegypti* were exposed to sublethal concentrations of the insect growth regulator, methoprene, and the glycogen content of pupae and surviving adults was compared and effects on adult longevity determined. The glycogen reserves in both male and female *Ae. aegypti* pupae were significantly reduced as a result of methoprene exposure. The longevity of adult females was also significantly reduced, but exposure affected neither the longevity nor the glycogen content of adult males. Adult sugar feeding increased the amount of glycogen in both treated and control females. The reduced longevity of adult females from larval methoprene treatment appeared not to be directly related to reduced glycogen, but rather reflected neuroendocrine abnormalities induced by this juvenile hormone analogue.

INTRODUCTION

The synthetic insect growth regulator, methoprene, is widely used in mosquito control. As an analogue of juvenile hormone, which regulates many basic physiological processes in insects, methoprene is believed to kill mosquitoes by interfering with normal neuroendocrine functions (Siddall 1976, Gordon and Burford 1984) and by inducing morphogenetic aberrations (Arias and Mulla 1975). Methoprene itself is not toxic to the insects, but the developmental abnormalities it causes leads to death (Siddall 1976). Unlike natural juvenile hormone, methoprene is not affected by degradative enzymes that normally break down juvenile hormone (Weirich and Wren 1973), and by suppressing juvenile hormone esterase activity (Downer et al. 1975), methoprene also extends hormonal activity beyond its normal limits.

In addition to its lethal effects on immature stages, methoprene also has sublethal effects on adult populations exposed to low doses as larvae (Bouchard and Wilson 1987, Robert and Olson 1989), and kills marginally nourished adults that are treated with it topically (Klowden and Chambers 1989). If larval exposure to low doses of methoprene can affect the physiological processes of surviving mosquito adults, their biting activity may be reduced even though these effects would not be reflected in the numbers of adults emerging. In this way, an equivalent degree of control might be accomplished with far less cost. Our objective in this study was to examine the effects of larval exposure to sublethal doses of methoprene on the longevity and glycogen content of surviving adult mosquitoes.

MATERIALS AND METHODS

Both larvae and adults of the UGAL strain of *Aedes aegypti* (Linn.) were maintained at 27°C. Larvae were reared on a diet consisting of brewer's yeast, lactalbumin hydrolysate and finely ground rat chow (1:1:1 by weight). Adults had access to 10% sucrose from cotton wicks.

S-methoprene was a gift from Zoecon Corporation (Dallas, TX). It was dissolved in acetone before serial dilutions were made in water. For each dilution, 10 early fourth instar larvae were transferred to 100 × 25 mm petri dishes containing 50 ml of water with the desired concentrations of methoprene and powdered Tetramin® fish food. Three groups of mosquitoes were exposed to each methoprene dose, and each experiment was repeated at least 3 times. The LC_{50} was determined by probit analysis (Statistical Analysis System, Cary, NC), and doses slightly above and below this LC_{50} used in subsequent experiments. After exposure to methoprene, 48 h pupae and newly emerged adult mosquitoes were sonicated and their total glycogen contents measured by the method of Van Handel (1985). A one-way analysis of variance was used to determine if significant differences existed in glycogen content due to methoprene treatment.

In longevity experiments, emerging adult males and females were maintained together in the same cage at 70–80% RH, with constant access to cotton wicks soaked with 10% sucrose. Mortality was determined daily.

RESULTS AND DISCUSSION

The concentration of methoprene that killed 50% of *Ae. aegypti* larvae (LC_{50}) was 0.13 ppb (slope = 2.08). When larvae were exposed to 0.1 and 0.2 ppb of methoprene, the longevity of the surviving adult females that were maintained on sucrose was reduced (Fig. 1), similar to the findings of Robert and Olson (1989) for *Culex quin-*

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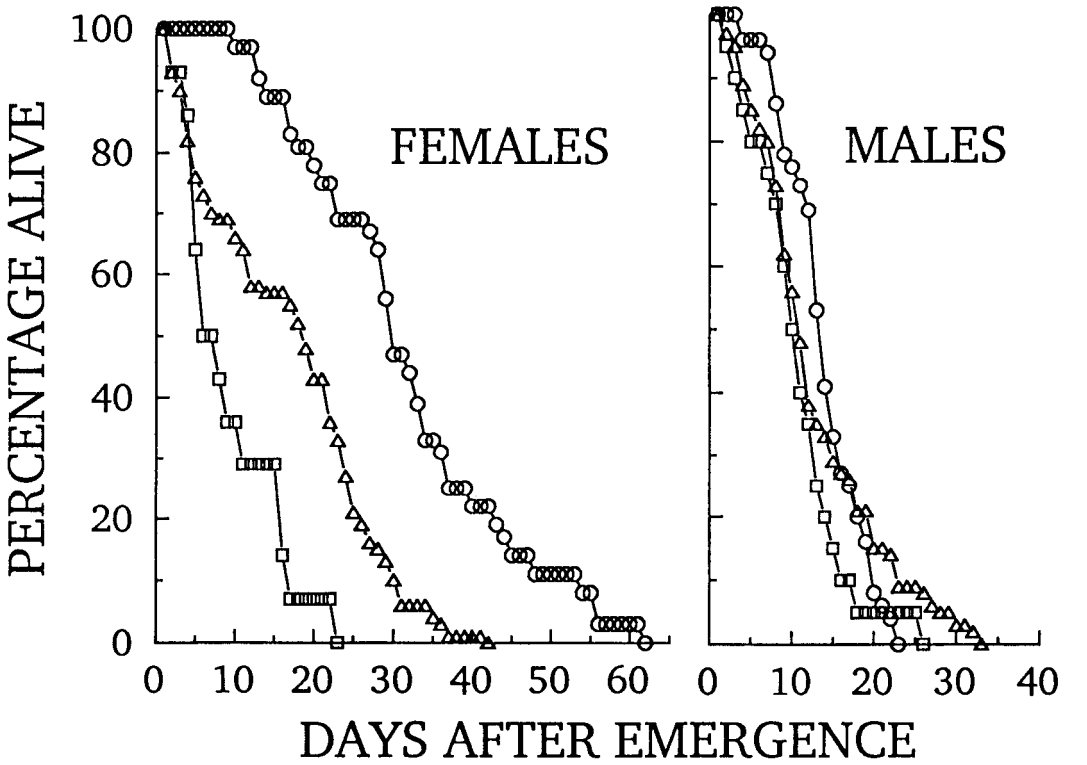

Fig. 1. Longevity of adult *Ae. aegypti* females (n = 101) and males (n = 137) that survived methoprene exposure as larvae. Circles: controls, not exposed to methoprene. Triangles: 0.1 ppb methoprene. Squares: 0.2 ppb methoprene.

Table 1. Mean glycogen content of 48 h male and female *Aedes aegypti* pupae developing from methoprene-treated larvae.

Larval methoprene concentration (ppb)	μg glycogen ± SE (n)	
	Males	Females
0	36.58 ± 1.99a* (58)	33.00 ± 0.88a (55)
0.1	26.27 ± 1.84b (60)	25.62 ± 1.21b (57)
0.2	23.34 ± 1.79b (46)	14.49 ± 1.39c (63)

* Means followed by the same letter within a column are not significantly different ($P > 0.05$).

quefasciatus Say. The effect was dose dependent, with 50% of the population dying after 6 days when exposed to the highest dose of 0.2 ppb, compared to 30 days for controls (Fig. 1). In contrast, larval methoprene exposure had no effect on the longevity of emerging adult *Ae. aegypti* males (Fig. 1).

Because previous reports suggest juvenile hormone and methoprene can influence the mobilization of glycogen (Downer et al. 1976, Gordon and Burford 1984, Klowden and Chambers 1989), we examined the glycogen reserves of pupae and adults that were treated as larvae. Both female and male *Ae. aegypti* pupae exposed as larvae to 0.1 and 0.2 ppb of methoprene showed a dose-dependent reduction in glycogen content (Table 1). At emergence, surviving adult females contained significantly ($P < 0.001$) less glycogen than untreated females, but there was no significant difference between the glycogen content of treated and untreated adult males (Table 2).

We then maintained adult females on sucrose for 3 days to determine whether glycogen pools were irreversibly affected by larval methoprene treatment. As shown in Table 2, after 3 days of feeding on 10% sucrose, levels of glycogen in adult female *Ae. aegypti* that were exposed to 0.1 or 0.2 ppb of methoprene as larvae did not differ significantly ($P > 0.2$) from untreated

Table 2. Glycogen content of adult *Aedes aegypti* treated with sublethal concentrations of methoprene as larvae.

Adult age	Larval methoprene concentration (ppb)	μg glycogen ± SE (*n*)	
		Males	Females
Newly emerged	0	18.19 ± 1.18a* (27)	16.00 ± 0.57b (106)
	0.2	17.12 ± 1.41a (29)	13.34 ± 0.48c (130)
3 days sugar-fed	0	ND	70.90 ± 3.47d (53)
	0.2	ND	68.13 ± 4.08d (42)

* Means followed by the same letters are not significantly different ($P > 0.05$). ND = no data.

controls in their levels of glycogen. It therefore appeared to be unlikely that methoprene affected adult female mortality by its effect on glycogen levels, because in our longevity experiments, adults were routinely provided with sucrose.

Although methoprene affected the glycogen content of both male and female *Ae. aegypti* pupae, only female adults showed a significant decline in glycogen (Table 2). This result is most likely due to differences in adult survival after methoprene treatment rather than differences in susceptibility between the sexes. Mortality due to methoprene treatment is usually expressed during the larval-pupal molt. As demonstrated by Robert and Olson (1989), more adult *Cx. quinquefasciatus* females than males emerge after sublethal methoprene exposure. Thus, we sampled all treated pupae, but only those adults that successfully emerged. Apparently, only those males with sufficient glycogen reserves were able to complete metamorphosis.

Juvenile hormone and its analogues have been shown to affect glycogen metabolism in insects (Wright and Rushing 1973, Downer et al. 1976, Klowden and Chambers 1989), but factors from the medial neurosecretory cells are also involved in mosquitoes (Van Handel and Lea 1970). The replenishment of glycogen reserves in *Ae. aegypti* after carbohydrate ingestion (Table 2) suggests that lower reserves are not directly responsible for increased adult mortality, but may reflect other physiological changes induced by methoprene.

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