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EFFECTS OF SUBLETHAL DOSAGES OF METHOPRENE ON *ANOPHELES DIRUS* SPECIES A AND B[1]

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ABSTRACT. *Anopheles dirus* species A and B individuals were exposed as 4th-stage larvae to sublethal concentrations of methoprene. Median lethal concentrations were 0.21 ppb for species A and 0.17 ppb for species B. When exposed to 0.10 ppb methoprene the sex ratio of species A changed from fewer males to more males; no effect was observed in the sex ratio of species B. Exposure to methoprene had no effect on wing length and survival of either species. Results indicate that exposure to methoprene significantly affected fecundity of both species of *An. dirus*.

INTRODUCTION

Anopheles dirus Peyton and Harrison is a mosquito species that is widely distributed throughout Southeast Asia and is one of the most important vectors of falciparum and vivax malaria (Rosenberg et al. 1990). Control of *An. dirus* adults is extremely difficult. The species is largely unaffected by indoor spraying of houses with DDT and fenitrothion because it is primarily exophilic (Prasittisuk 1985). Possible control of immature stages has not been seriously considered because larvae inhabit small, temporary ground pools in forested areas, often in inaccessible regions in hills along international borders (Rattanarithikul and Panthusiri 1994). Consequently, larval habitats are very difficult to locate and treat with larvicides.

Characteristics of pellet and granule formulations of methoprene, such as its ability to be used as a sustained release larvicide (Linthicum et al. 1989, Kramer and Beesley 1991), its ability to retain activity for several months during pretreatment in dry areas (Logan et al. 1990), and its potential ability to penetrate canopy covers, may make it suitable for aerial application as a larvicide for *An. dirus* in areas where malaria is endemic. In addition to the lethal effects of methoprene, sublethal effects on adult mosquito populations exposed to low doses as larvae also have been observed (Robert and Olson 1989, Sawby et al. 1992, Beehler and Mulla 1993).

In the present study, specimens of *An. dirus* species A and B were exposed in the laboratory as 4th-stage larvae to sublethal concentrations of methoprene. Possible effects on adult sex ratio, wing length, survival, feeding success, oviposition, and egg hatching were measured.

MATERIALS AND METHODS

Mosquitoes: Anopheles dirus species A were from a colony established from Chonburi, Thailand, in 1968, that was maintained using forced-mating techniques. This colony has been periodically outbred with field-collected specimens. The most recent outcross occurred 1 month before the start of this study. *Anopheles dirus* species B were from a self-mating colony established from Sabah, Malaysia, in 1978. Both colonies were reared at 27 ± 2°C, 75–80% RH, in natural light for multiple generations.

Methoprene: Altosid® (4% methoprene) sustained-release pellets used in this study were provided by Zoecon Corporation, Dallas, TX. Pellets were dissolved in acetone before serial dilutions were made in water (Robert and Olson 1989). For each dilution, approximately 100 early 4th-stage larvae were placed in 6 plastic pans (25.4 × 30.5 × 5.1 cm) containing 1.5 liters of water and either 0, 0.01, or 0.10 ppm concentrations of methoprene. An abundant amount of a crushed locally purchased fish food was added every afternoon to each pan and the excess infusion removed each morning. Each species was exposed to each methoprene dose, and each exposure was repeated 7 times.

Experimental procedures: After exposure of 4th-stage larvae to methoprene for 36–48 h, pupae were removed from plastic pans and placed

[1] The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to the *Guide for Use of Laboratory Animals*, NIH Publication 86-23, 1985 ed.

Table 1. Sex ratio in *Anopheles dirus* adults and wing lengths in ovipositing females after exposure of 4th-stage larvae to sublethal doses of methoprene.

Methoprene concentration (ppb)	Mean sex ratio ± SE (male : female)		Mean wing length (mm) ± SE	
	Species A	Species B	Species A	Species B
0	0.75 ± 0.21a[1]	0.37 ± 0.01	3.03 ± 0.12	3.11 ± 0.05
0.01	0.76 ± 0.10a	0.24 ± 0.11	2.99 ± 0.11	3.13 ± 0.04
0.10	1.50 ± 0.10b	0.35 ± 0.20	2.95 ± 0.10	3.12 ± 0.06

[1] Means within a column followed by different letters are significantly different (ANOVA, $P < 0.05$).

directly in a paper cup in a 0.3-m^3 wire mesh cage. Percent mortality of pupae was determined by counting the number of adults successfully emerging and the number of dead pupae and adults. The sex of all emerging adults was recorded. In each trial adult survival was measured by placing 10 of the male and 10 of the female adults that emerged in each of 2 0.25-liter plastic cups covered with nylon mesh and counting the number dead each day until all individuals had died. To measure survival under stressful and normal conditions cups were provided with either a water or a sucrose source and placed at room temperature (26°C). The remainder of the emerging adult females were exposed 7 days after emergence to an anesthetized adult golden Syrian hamster in a 0.3-m^3 cage. The number feeding, based upon the appearance of any amount of blood in the abdomen, was recorded. All *An. dirus* species A (after force-mating) and species B individuals that had fully engorged were placed individually in 25-ml glass bottles containing 5-cm strips of filter paper and 5 ml of water and allowed to oviposit. The number of eggs oviposited and the number hatching was recorded. The wing length of all mosquitoes that oviposited was measured.

Statistical analysis: The median lethal concentration (LC_{50}) of methoprene for each species was determined by probit analysis (SPSS Inc. 1993). Estimates of mean survival time in days were determined by Kaplan–Meier survival analysis (SPSS Inc. 1993). Comparisons of mean sex ratio, wing length, and number of eggs oviposited were made using ANOVA. Comparisons of pupal mortality, feeding success, oviposition, and egg hatching were made using multiple contingency table tests for independence (Centers for Disease Control 1994).

RESULTS AND DISCUSSION

The LC_{50} of methoprene for *An. dirus* was 0.21 ppb for species A and 0.17 ppb for species B. The LC_{50} was similar to that reported for *Aedes aegypti* (Linn.) (Sawby et al. 1992) and *Culex quinquefasciatus* Say (Robert and Olson 1989). Exposure of larvae to concentrations of methoprene of 0.01 and 0.10 ppb was equivalent to an LC_{31} and LC_{40} in species A and an LC_{30} and LC_{41} in species B, respectively. There was significantly more mortality in specimens exposed to methoprene than in those of the control group (chi-square, $P < 0.0001$). Exposure of species A to 0.10 ppb of methoprene significantly changed the sex ratio from fewer males to more males (Table 1, chi-square, $P < 0.01$), but had no effect on species B. Conversely, Robert and Olson (1989) observed a significant increase in the proportion of females of *Cx. quinquefasciatus* exposed to methoprene. Wing length of ovipositing females was not affected by exposure to methoprene in either species (Table 1). Methoprene has been documented to cause both longer and shorter wing length in *Culex pipiens* Linn. and *Cx. quinquefasciatus* (Kelada et al. 1981, Robert and Olson 1989) at concentrations near the LC_{50}. No change in survival in groups given water or sucrose of either species was observed following exposure to methoprene (Table 2). At slightly higher concentrations of methoprene reduced survival has been reported for *Cx. quinquefasciatus* (Robert and Olson 1989) and *Culex tarsalis* coq. (Arias and Mulla 1975).

Although methoprene did not have any effect on the number of *An. dirus* species A feeding (Table 3), feeding was significantly reduced in species B by 10% and 25% after exposure to 0.01 and 0.10 ppb of methoprene, respectively (Table 3, chi-square, $P < 0.01$). Similarly, exposure to methoprene did not affect the number of fully engorged species A females that oviposited; however, the number of species B females that oviposited was reduced by approximately 19% and 22% after exposure to 0.01 and 0.10 ppb of methoprene, respectively (Table 3, chi-square, $P < 0.001$). There was no effect on the mean number of eggs from ovipositing females of either species. Methoprene has been found to reduce the number of *Cx. quinquefasciatus* eggs produced (Robert and Olson 1989).

Table 2. Survival of *Anopheles dirus* adults after exposure of 4th-stage larvae to sublethal doses of methoprene.

Nutrient source	Methoprene concentration (ppb)					
	Species A			Species B		
	Control	0.01	0.10	Control	0.01	0.10
Water						
Female	19[1] (15–23)	19 (15–23)	17 (14–20)	3 (2–4)	2 (2–3)	2 (2–3)
Male	2 (1–3)	5 (3–7)	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–2)
Sucrose						
Female	37 (32–42)	33 (28–37)	35 (30–39)	13 (10–15)	16 (14–18)	17 (15–19)
Male	26 (22–30)	35 (30–39)	27 (23–31)	17 (15–20)	15 (13–18)	14 (11–16)

[1] Mean survival time in days (95% confidence interval).

Although there was no effect on hatching rate of eggs for species B, egg hatching for species A was reduced more than 11% and 16% after exposure to 0.01 and 0.10 ppb methoprene, respectively (Table 3, chi-square, $P < 0.01$). Reduced egg hatching has been demonstrated for *Cx. quinquefasciatus* (Robert and Olson 1989) but not for *Cx. pipiens* (Kelada et al. 1981) or *Ae. aegypti* (Firstenberg and Sutherland 1981). Exposure of larvae to sublethal levels of methoprene significantly reduced the fecundity of *An. dirus* species A by lowering the egg hatching rate and of species B by inhibiting feeding and oviposition. The results of this laboratory study using colonies that had been maintained for a long period of time may not reflect what might occur using fresh stocks. However, our data suggest that methoprene may be efficacious for control of *An. dirus* at sublethal doses. This information may be important in designing effective mosquito control strategies for malaria control in Southeast Asia.

Table 3. Feeding and fecundity of *Anopheles dirus* species A and species B adults after exposure of 4th-stage larvae to sublethal doses of methoprene.

Methoprene concentration (ppb)	Percent fed[1] (no. tested)	Percent ovipositing (no. tested)	Mean number of eggs (±SE)	Percent hatching (no. tested)
Species A				
0	54.6 (227)	54.5 (88)	77.7[2] (55)	74.5a[3] (4,504)
0.01	49.4 (170)	65.3 (72)	75.9 (6.4)	63.0b (3,492)
0.10	55.6 (63)	65.4 (26)	78.9 (9.6)	57.9c (1,342)
Species B				
0	66.4a (366)	60.6a (148)	87.2 (38.0)	94.0 (5,234)
0.01	56.1b (269)	41.3b (143)	101.1 (38.9)	95.0 (3,335)
0.10	41.3c (218)	38.3b (112)	90.3 (32.7)	90.0 (3,703)

[1] Includes partially and fully engorged specimens.
[2] Means calculated only for females that did oviposit.
[3] Percentages followed by different letters are significantly different (multiple 2 × 2 contingency tables, $P < 0.01$).

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