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ACUTE AND SUBLETHAL EFFECTS OF (*S*)-METHOPRENE ON SOME AUSTRALIAN MOSQUITOES

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ABSTRACT. Laboratory bioassays were used to determine the efficacy of (*S*)-methoprene against 7 species of Australian mosquitoes. The 90% lethal concentration (LC_{90}) ranged from 0.17 ppb for *Aedes vigilax* to 6.54 ppb for *Culex sitiens*. The survival of adults exposed as larvae to 2 sublethal dosages of (*S*)-methoprene was compared to a control group. Little effect was noted for *Cx. sitiens* and *Culex annulirostris*. However, survival of male and female *Ae. vigilax* was significantly reduced and appeared to be dose related. Bloodfeeding success of female *Ae. vigilax* was also significantly reduced.

INTRODUCTION

The insect growth regulator methoprene has been successfully used to control many species of mosquitoes, with minimal nontarget toxicity (for a review see Ross et al. 1994a, 1994b). Methoprene occurs in 2 forms, the (*R*) and (*S*) isomers, with (*S*)-methoprene having the greatest insecticidal activity. (*S*)-methoprene is available in many formulations. Liquid formulations (ALTOSID® Liquid Larvicide [A.L.L.]) provide control over a limited period, whereas sustained-release formulations such as ALTOSID® Pellets and XR Briquets can control mosquitoes for several months (Kramer et al. 1993, Ross et al. 1994a).

We conducted laboratory bioassays to determine the efficacy of A.L.L. against 7 common Australian mosquitoes. *Aedes aegypti* (L.) is a vector of dengue in northern Queensland (Cleland et al. 1918); *Aedes funereus* (Theobald), *Aedes notoscriptus* (Skuse), *Aedes vigilax* (Skuse), *Culex annulirostris* Skuse, and *Culex sitiens* Wiedemann are all known or potential vectors of Ross River virus (Russell 1994; Ritchie et al., in press), with *Cx. annulirostris* a vector of Murray Valley encephalitis (McLean 1953) and probably Japanese encephalitis (Ritchie et al. 1997). *Anopheles farauti* (Laveran) *s.l.* is considered a major vector of malaria throughout Australasia, including Queensland north of the 19th parallel (Lee et al. 1989).

Poor application of methoprene, coupled with loss of efficacy of sustained-release formulations, can lead to adult emergence. Nonetheless, adults from larvae exposed to sublethal concentrations of methoprene can be adversely affected. Sawby et al. (1992) found that sublethal doses of (*S*)-methoprene decreased survival of adult *Ae. aegypti*. However, adult survival was not significantly influenced in *Anopheles dirus* Peyton and Harrison, although bloodfeeding and oviposition were depressed (Sithiprasasna et al. 1996). Methoprene is also documented to affect body size (wing length) and sex ratio (Robert and Olson 1989). We investigated the effects of sublethal amounts of (*S*)-methoprene on adult longevity in *Ae. vigilax, Cx. annulirostris,* and *Cx. sitiens,* and on bloodfeeding success in *Ae. vigilax,* the primary target for mosquito control programs in coastal Australia.

MATERIALS AND METHODS

Laboratory bioassays: Laboratory bioassays were used to determine the effectiveness of (*S*)-methoprene (A.L.L., 5% active ingredient) against 7 common Australian mosquitoes. *Aedes aegypti, Ae. notoscriptus, Ae. vigilax, An. farauti, Cx. annulirostris,* and *Cx. sitiens* were from colonies maintained at the Queensland Institute of Medical Research, whereas *Ae. funereus* eggs were obtained from wild mosquitoes collected in Cairns, Queensland. Twenty 3rd-instar larvae were exposed to different concentrations of A.L.L. in 200 ml of deionized water within a capped, 250 ml Schott glass (*Ae. funereus* and *Cx. sitiens,* and *Ae. vigilax* larvae were reared in 16 and 33% seawater, respectively). Preliminary trials indicated that survival of *Ae. funereus* and *An. farauti* was improved using 100 ml of water; thus 100 ml of water was used in bioassays with these species. A control using 100 ppb of the A.L.L. emulsion without (*S*)-methoprene was included in each trial. All trials featured 3–4 dose replicates. Larvae were fed a slurry of fish food and liver powder (*An. farauti* were fed on a powder diet that spread across the water surface) and reared at 28°C with a 14:10 light : dark cycle.

The % emergence inhibition (%EI) was calculated as:

$$\%EI = 100 \times [1 - (\text{number of emerging adults}/20)].$$

Because the Schott jar was capped, emerged adults included eclosed adults that were incapable of flight. For each trial, %EI was corrected for control mortality using Abbott's formula (Abbott 1925). Probit analysis (Finney 1971) was used to determine the respective median lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) of (*S*)-methoprene using Probit 5 for Windows® (P. Gil-

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Table 1. Lethal concentration of (*S*)-methoprene[1], in parts per billion (ppb), to kill 50 (LC_{50}) and 90% (LC_{90}) of the larvae of 7 Australian mosquitoes.[1]

Mosquito species	No. tested[2]	Dose range (ppb AI)	LC_{50} (95% CI)	LC_{90} (95% CI)	Minimum lethal dose[3]
Aedes aegypti	420	0.156–10.0	0.397 (0.318–0.496)	1.308 (0.963–1.779)	2.50
Aedes funereus	430	0.016–2.5	0.072 (0.051–0.103)	0.685 (0.536–1.076)	2.5
Aedes notoscriptus	360	0.031–4.0	0.359 (0.288–0.447)	1.117 (0.824–1.513)	4.0
Aedes vigilax	560	0.004–0.50	0.022 (0.017–0.028)	0.167 (0.105–0.265)	0.50
Anopheles farauti	720	0.016–4.0	0.057 (0.040–0.080)	0.986 (0.587–1.657)	4.0
Culex annulirostris	720	0.003–1.25	0.089 (0.076–0.105)	0.341 (0.262–0.443)	0.625
Culex sitiens	780	0.039–40.0	1.124 (0.924–1.367)	6.544 (4.944–8.664)	40.0

[1] As determined by probit analysis (Finney 1971).
[2] Excluding controls.
[3] The dose (in ppb) inclusive and beyond which all test larvae died.

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The effect of sublethal dosages of (S)-methoprene: The impact of sublethal dosages of (*S*)-methoprene on the survival of adult *Ae. vigilax, Cx. annulirostris,* and *Cx. sitiens* was determined by exposing 300–2,000 3rd-instar larvae to A.L.L. at concentrations of 0 (control) and the respective LC_{50} and LC_{90} determined from the laboratory bioassays. Pupae were placed in 60-ml beakers placed in a 5-liter cardboard bucket (Kentucky Fried Chicken Family Size®) covered with screen and a moist paper towel to maintain high humidity. The effective %EI was determined as described earlier, but because the emergence jar was open within the bucket, dead adults on the water surface were not included in the emerged adults total. The beakers were removed from the bucket and emerged adults were maintained at 28°C with a 14:10 light:dark cycle and given access to 10% sucrose and sliced apple. Dead adults were removed and tallied daily. For each sex of all species tested, survival functions for the 3 treatments were compared by the log-rank test (Armitage and Berry 1987) using PROC LIFETEST in SAS (SAS Institute Australia Pty. Ltd., Private Bag No. 52, Lanecove, New South Wales 2066 Australia; Release 6.13 licensed to the Tropical Public Health Unit).

For the bloodfeeding experiment, 15 adult female *Ae. vigilax* from larvae exposed to 0.12 ppb (*S*)-methoprene (*ca.* LC_{80}) or a control group were placed in a 400-ml glass beaker (covered with cloth gauze) *ca.* 4 days after emergence. Mosquitoes were starved for 12 h, then, at dusk, offered a human blood meal for 6 min. The experiment was replicated 9 times. The percentage (arcsine transformed) of bloodfed mosquitoes for each group was compared by a Student's *t*-test.

RESULTS AND DISCUSSION

Bioassays of (S)-methoprene: Most of the mosquitoes were very susceptible to (*S*)-methoprene, with LC_{90}s generally < 2 ppb (Table 1). *Aedes vigilax* was especially susceptible, with an $LC_{90} < 0.2$ ppb. Vector control programs have had great success controlling this mosquito with (*S*)-methoprene in southeast Queensland, with treatments of ALTOSAND® (Biorational Resources, Scarborough, Queensland, Australia) (0.4% AI) at 2–3 kg/ha providing control of *Ae. vigilax* for 7–10 days (S. A. Ritchie, D. McGinn, and N. Woods, unpublished data). However, *Cx. sitiens* is not as susceptible to (*S*)-methoprene, with an LC_{90} 39 times greater than that for *Ae. vigilax.* Field studies with ALTOSAND® and ALTOSID® Pellets confirm this (S. A. Ritchie, unpublished data) and suggest that treatment failures are likely to occur from March to May when *Cx. sitiens* is most abundant in the salt marsh.

The effect of sublethal doses of (S)-methoprene: Sublethal doses of (*S*)-methoprene significantly ($P < 0.01$) affected the survival of adult *Ae. vigilax* (Table 2). Mortality of *Ae. vigilax* males and females was directly related to dosage. Generally, sublethal doses of (*S*)-methoprene did not significantly affect the survival of the *Culex* tested except for male *Cx. annulirostris* (Table 2). Interestingly, sublethal effects seemed to be related to susceptibility in the bioassay. Larval *Ae. vigilax* were affected at relatively lower doses, with surviving adults suffering reduced survival, whereas larval *Cx. sitiens* had relatively low susceptibility with no noticeable effects on adult survival.

In *Ae. vigilax,* (*S*)-methoprene significantly affected bloodfeeding success. Adult females surviving 0.12 ppb of (*S*)-methoprene, which prevented eclosion of 89.2% of larvae, bloodfed at a significantly lower ($t = 5.34$, df = 16, $P < 0.001$) rate (mean ± SD = 23 ± 15%) than the control group (65 ± 16%).

These data, particularly at the higher doses, suggest that *Ae. vigilax* exposed to nearly lethal doses of (*S*)-methoprene may be a minimal pest and disease threat. Bloodfeeding success may be reduced, and the survival of females may be insufficient to develop viremia (*ca.* 4 days at 28°C, for Ross River virus [Kay 1982]). However, careful field studies of this phenomenon are needed before the findings of the study reported here are applicable to operational situations.

Table 2. Mean (± SE) survival in days of adult mosquitoes exposed to different doses of (*S*)-methoprene (ALTOSID® Liquid Larvicide).[1]

Species		Dose (ppb AI)		
Aedes vigilax		0	0.10	0.12
	%EI	5.0%	40.2%	89.2%
	Male	74, 12.3 ± 0.5a, 23	480, 6.1 ± 0.1b, 20	71, 2.3 ± 0.2c, 7
	Female	82, 17.3 ± 0.75a, 31	687, 9.0 ± 0.13b, 21	71, 5.0 ± 0.2c, 10
			Dose (ppb AI)	
Culex annulirostris		0	0.5	1.2
	%EI	13.4%	43.7%	71.2%
	Male	71, 20.4 ± 1.4a, 47	92, 26.8 ± 1.2b, 51	50, 17.6 ± 1.1c, 28
	Female	163, 28.6 ± 0.8a, 49	53, 28.0 ± 1.8a, 54	37, 27.7 ± 1.7a, 42
			Dose (ppb AI)	
Culex sitiens		0	4	10
	%EI	1.6%	46.0%	85.1%
	Male	314, 223 ± 0.5a, 50	115, 21.3 ± 0.7a, 49	17, 19.5 ± 2.4a, 41
	Female	351, 27.7 ± 0.6a, 62	120, 25.5 ± 1.0a, 55	31, 23.4 ± 2.5a, 45

[1] %EI = % emergence inhibition. Data for male and female are given as *n*, mean ± SE days survived, maximum days survived. Means followed by a different letter are significantly different ($P < 0.05$) by the log-rank test (Armitage and Berry 1987).

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