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# ACUTE TOXICITY OF SELECTED PESTICIDES TO THE ESTUARINE SHRIMP *LEANDER TENUICORNIS* (DECAPODA: PALAEMONIDAE)

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ABSTRACT. The shrimp *Leander tenuicornis* is abundant in southeastern Queensland intertidal marsh pools and was chosen as an indicator species for toxicological studies with pesticides. Acute toxicity to this crustacean of temephos and 3 pesticide compounds under evaluation for registration in Australia (*Bacillus thuringiensis* var. *israelensis, s*-methoprene, and pyriproxyfen) was tested in 96-h laboratory trials. Temephos was the most toxic compound, with a median lethal concentration ($LC_{50}$) of 0.01 ppm (0.33 times the estimated field concentration [EFC] for a 15-cm-deep pool). *s*-Methoprene was the least toxic compound, with an $LC_{50}$ of 14.32 ppm (1,790 times the EFC). *Bacillus thuringiensis* var. *israelensis* and pyriproxyfen produced $LC_{50}$ values of $60.9 \times 10^6$ ITU (176 times the EFC) and 0.098 ppm (12.25 times the EFC), respectively.

## INTRODUCTION

Knowledge of the toxicities to target and nontarget organisms of pesticides used for mosquito control purposes is essential for responsible management. For the past 20 years in eastern Australia, salt-marsh mosquito control programs have been reliant on the application of various formulations of Abate® (temephos) to control larvae of *Aedes vigilax* (Skuse) and *Culex sitiens* Wiedemann (Kay et al. 1973). Surprisingly, despite the widespread and prolonged usage of temephos, there is little published information available on the susceptibility of Australian nontarget species to this compound (Kay et al. 1973, Gehrke 1988, Mortimer and Hughes 1991, Mortimer and Chapman 1995) and much of that has only become available *a posteriori.* There are growing environmental concerns over usage of temephos, and mounting evidence of mosquito resistance to its use (Cousineau 1992). Alternative compounds are now being evaluated for mosquito control purposes. These include various formulations of the insect growth regulators, *s*-methoprene and pyriproxyfen; and the bacterial larvicide *Bacillus thuringiensis* serovar. *israelensis* de Barjac (*B.t.i.*).

To provide an estimate of the concentration of pesticide that causes direct, irreversible harm to nontarget species, a series of 96-h acute toxicity tests were designed. From survey data in regional council treatment areas in southeastern Queensland, the palaemonid shrimp *Leander tenuicornis* Say was selected for laboratory bioassay because it is abundant in the shallow estuarine and wetland fauna around Moreton Bay and Pumicestone Passage (Wadley 1978), it has known importance in estuarine food webs (Wadley 1978), there is a coincidence of maximum breeding season with that of mosquitoes and hence dosing programs (J. G. Greenwood, unpublished data), and it is readily maintained in laboratory culture.

## MATERIALS AND METHODS

*Collection, maintenance, and identification of test species:* Late-juvenile to adult shrimp were collected from salt-marsh pools near Coomera Marina (27°54′S, 153°17′E) in southeastern Queensland, using a 25 × 25 × 45-cm, 2-mm-mesh bait fish trap (Mossop's Tackle Pty. Ltd., Brisbane, Queensland, Australia). Collected specimens were placed in aerated habitat water for transport to laboratories in Brisbane. Additional habitat water was also collected for subsequent maintenance and experimental purposes. To remove detritus, all water for experimentation and maintenance of test animals was passed through a 100-μm-mesh net prior to use. In the laboratory, shrimp were transferred into 24 × 22 × 46-cm aerated aquaria for a 3–4-day acclimation period. Recently hatched *Artemia salina* nauplii (Marine Laboratory, Hayward, CA, USA) and Wardley's Goldfish Food (Wardley Corporation, Seaucus, NJ, USA) were provided as food. *Leander tenuicornis* was identified according to the description in Wadley (1978).

*Test animals:* To minimize variability of response to test material, adult shrimp of uniform length were tested. Measurements of 20 (10 male and 10 gravid female) freshly killed individuals were taken with Vernier callipers and recorded to the nearest 0.05 mm. The measurements were based on carapace length, which is defined as the distance measured along a dorsal median line drawn from the posterior margin of the orbit of the eye socket to the posterior margin of the carapace. Only individuals that were active after the 3–4-day acclimation period were used in trials.

*Pesticides evaluated:* In order to evaluate the ef-

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Table 1. Concentrations of pesticides causing 50% mortality of *Leander tenuicornis* after 96-h exposure. Abiotic conditions are listed.

| Active ingredient | EFC[1] | $LC_{50}$[2] |
|---|---|---|
| Temephos (ppm) | 0.03 | 0.0112 (0.0099, 0.0126) |
| *Bacillus thuringiensis* var. *israelensis* (ITU)[4] | $0.346 \times 10^6$ | $60.9 \times 10^6$ ($49.5 \times 10^6$, $75.0 \times 10^6$) |
| *s*-Methoprene (ppm) | 0.008 | 14.32 (12.39, 22.42) |
| Pyriproxyfen (ppm) | 0.008 | 0.098 (0.081, 0.117) |

[1] Estimated field concentration of active ingredient for 15-cm-deep pools.
[2] Median lethal concentration; values in parentheses are the 95% lower and upper confidence limits.
[3] *P* refers to the probability corresponding to maximum likelihood chi-square statistic for goodness of fit of the model.
[4] ITU = International Toxic Units.

fects of pesticides utilized in field applications, we tested Abate 100E® (active ingredient [AI]: 10% temephos applied at 0.1 kg AI/hectare, Cyanamid Australia Pty. Ltd., Baulkham Hills, New South Wales, Australia), VectoBac 12AS® (AI: 1,200 International Toxic Units [TTU]/mg *B.t.i.* applied at 288 mg AI/hectare, Hoechst Schering AgrEvo Pty. Ltd., Pennant Hills, New South Wales, Australia), Altosid Liquid Larvicide® (AI: 20% *s*-methoprene applied at 0.06 kg AI/hectare, Sandoz Ltd., Dallas, (TX, USA), and Sumilarv® (AI: 2% pyriproxyfen applied at 0.06 kg AI/hectare, Hoechst Schering AgrEvo Pty. Ltd., Pennant Hills, New South Wales, Australia).

*Acute toxicity trials:* Static exposure assays were designed and implemented according to criteria specified by Rand and Petrocelli (1985) for acute toxicity testing of macroinvertebrates. In these assays, the test animals were exposed to serial dilutions of a larvicide in filtered habitat water, with no change of water for the duration of the assays. Three replicates each of 20 late-juvenile to adult specimens were introduced into 20 × 20 × 30-cm (12-liter) glass aquaria containing 5 liters of test concentration. Three negative control containers holding 20 test specimens each in habitat water without pesticide were used for each bioassay. A minimum of 10 dosages was tested for each pesticide. Test specimens were individually removed from the holding aquaria and distributed randomly among the test containers. To minimize variability due to nutritional and metabolic condition, shrimps were not fed for 24 h prior to, or during testing. As abiotic factors can affect the toxicity of a substance (Cooney 1995), salinity (mg/liter), pH, water temperature, and turbidity were measured using a portable field laboratory (Horiba Ltd., Kyoto, Japan). The assays were conducted at 25°C under a light: dark cycle of 12:12 hours. Death, or the lack of reaction to gentle prodding with a glass pipette, was the measured deleterious response. The numbers surviving were counted at 24-h intervals for 96 h and dead animals were removed from the test containers at each evaluation.

*Analysis of data—toxicity trials:* Probit models were used to model mortality as a function of pesticide dose. To avoid infinite logarithmically transformed values, zero concentrations were analyzed as concentrations of 0.000001 ppm. Approximately linear plots of the probit values by log (dose) indicated that the assumptions associated with fitting these probit models were met. The relationship between pesticide concentration and the percentage of exposed organisms affected was determined, and concentration–mortality curves were plotted. The SPSS-PC+ version 4.0 PROBIT procedure (Norusis 1990) was used for these analyses. The median lethal concentration ($LC_{50}$) values and associated 95% confidence intervals are presented.

## RESULTS

A mean length (±SD) of 2.41 (±0.16) cm was measured for *L. tenuicornis.* The probit analyses of toxicities for these pesticides against *L. tenuicornis,* and the abiotic conditions of testing are summarized in Table 1. The estimated field concentration (EFC) of active ingredient in 15-cm-deep pools for the pesticides evaluated is also presented in Table 1. Concentration–response curves for *L. tenuicornis* exposed to the selected pesticides for 96 h are presented in Fig. 1. Temephos was the most toxic compound tested against *L. tenuicornis,* with an $LC_{50}$ value of 0.0112 ppm. The $LC_{50}$ value of 0.0112 was approximately one-third of the EFC. *s*-Methoprene was the least toxic compound, with an $LC_{50}$ value of 14.32 ppm. This $LC_{50}$ value is approximately 1,790 times the EFC. With an $LC_{50}$ value of 0.098 ppm, pyriproxyfen was 146 times more toxic to *L. tenuicornis* than was *s*-methoprene and this represented 12.25 times the EFC. VectoBac 12AS exhibited low acute toxicity to *L. tenuicornis,* with an $LC_{50}$ value that was 176 times the EFC.

## DISCUSSION

Nontarget species such as *L. tenuicornis* are important members of the food chain (Morton et al.

Table 1. Continued.

| Slope (SE) | $P^3$ | Abiotic characteristics (mean ± SD) Salinity (ppk) | pH | Dissolved oxygen (mg/liter) | Turbidity | Temperature (°C) |
|---|---|---|---|---|---|---|
| 4.050 (0.369) | 0.262 | 40.0 | 7.8 | 3.9 (±0.3) | 0 | 25.0 |
| 4.459 (0.366) | <0.001 | 40.0 | 7.8 | 2.7 (±0.2) | 0 | 25.0 |
| 1.122 (0.114) | <0.001 | 39.0 | 7.9 | 2.8 (±0.4) | 0 | 25.0 |
| 3.491 (0.387) | 0.135 | 27.5 | 7.6 | 3.2 (±0.6) | 0 | 25.0 |

1988), and we recommend the use only of pesticides with proven low toxicity to nontarget species. Our results with respect to acute toxicity show that *s*-methoprene and *B.t.i* can be safely applied in situations where *L. tenuicornis* is present. For these products, the $LC_{50}$ values show that no mortality of this nontarget species is likely at the levels applied in control programs. Although it is acknowledged that laboratory tests may not provide a true indication of what will occur in the field, the dosages tested for *B.t.i.* and *s*-methoprene were sometimes far in excess of estimated field dosages applied aerially. Also, toxicity in these tests was not moderated by tidal flushing or sorption to organic matter (Cooney 1995). Subtle chronic effects are often difficult to measure (Cooney 1995). However, because *L. tenuicornis* tolerated excessively high dosages, one may hypothesize from these studies that no chronic toxicity occurs.

The $LC_{50}$ values obtained for *s*-methoprene and pyriproxyfen indicate that large differences in toxicity to nontarget fauna can occur between insect growth regulator products. In contrast to *s*-methoprene, pyriproxyfen should be applied with caution, as an $LC_{50}$ value of only 12.25 times the EFC does not allow a great margin for error. Consequently,

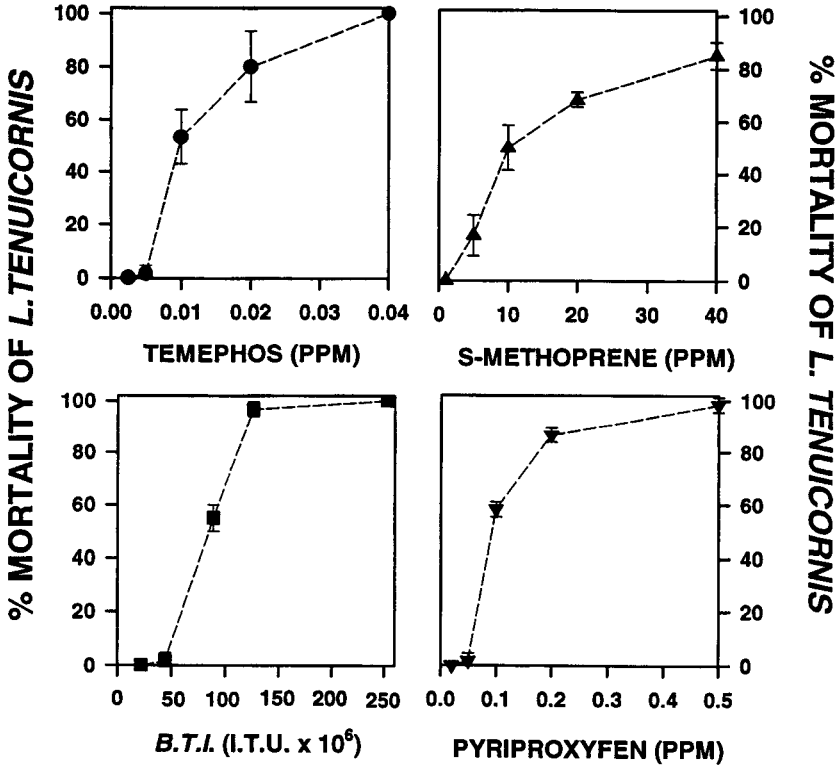


Fig. 1. Concentration–response curves for the shrimp *Leander tenuicornis* exposed to temephos, *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), *s*-methoprene, and pyriproxyfen for 96 h.

we would encourage long-term or indirect effect studies on this insect growth regulator. Also, it would be essential that aerial applications of pyriproxfen be of uniform dosage, which currently is not being achieved with any product used.

Without historical data on shrimp abundance in salt-marsh habitats in southeastern Queensland, the true impact of usage of temephos is impossible to gauge. However, based on the $LC_{50}$ values obtained in this study, we feel that a caution against further usage is implicit in this paper.

The toxicological data provided by this study should provide some assurance to mosquito control personnel and environmental managers responsible for salt-marsh habitats in eastern Australia. The wide differential between the EFC and $LC_{50}$ values for *s*-methoprene and *B.t.i.* should help allay the recent concerns of commercial shrimp aquaculturists that these newer products may be dangerous to their crustaceans.

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