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ACUTE TOXICITY OF SELECTED PESTICIDES TO THE PACIFIC BLUE-EYE, *PSEUDOMUGIL SIGNIFER* (PISCES)

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ABSTRACT. Because the larvivorous fish *Pseudomugil signifer* is native to southeastern Queensland and is abundant in shallow estuarine habitats, intertidal marshes, wetland habitats, and freshwater streams, it was chosen as an indicator species for toxicologic studies with pesticides. Acute toxicity studies with 2 organophosphorus pesticides (pirimiphos-methyl and temephos) and 3 alternate compounds under evaluation for registration in Australia (*Bacillus thuringiensis* var. *israelensis, s*-methoprene, and pyriproxyfen), were tested in 96-h laboratory trials. Pirimiphos-methyl was the most toxic compound, with a median lethal concentration (LC_{50}) of 0.091 ppm (0.3 times the estimated field concentration [EFC] for a 15-cm-deep pool). Temephos had an LC_{50} value of 0.594 ppm (9.9 times the EFC). *Bacillus thuringiensis* var. *israelensis* and pyriproxyfen produced LC_{50} values of 6.1×10^{11} International Toxic Units (477 times the EFC) and 0.854 ppm (106 times the EFC), respectively. *s*-Methoprene was the least toxic compound, with no mortality recorded at 500 times the EFC.

KEY WORDS Pesticides, non-target, toxicology, *Pseudomugil*, organophosphorus, *s*-methoprene, *B.t.i.*, pyriproxyfen

INTRODUCTION

An increasing range of compounds is being used in attempts to control larval mosquitoes and biting midges in aquatic habitats, often with hazardous or unknown effects on associated nontarget species (Mulla et al. 1978, Hershey et al. 1995, Pierce et al. 1996). Hence, the Australian Local Authorities Research Committee is developing data on the toxicities to target and nontarget organisms of pesticides used for control purposes. These data are essential for responsible management.

Surprisingly, despite the widespread use of pesticides, little published information is available on the susceptibility of Australian nontarget species to these compounds (Gehrke 1988, Mortimer and Hughes 1991, Mortimer and Chapman 1995, Brown et al. 1996), and much of that has only become available after the fact. Environmental concerns over the use of organophophorus larvicides are growing, and evidence of mosquito and midge resistance to temephos is mounting (Cousineau 1992; H. Standfast, personal communication). Alternative compounds are now being evaluated for mosquito and midge control purposes. These include various formulations of the insect growth regulators (IGRs) *s*-methoprene and pyriproxyfen and the bacterial larvicide *Bacillus thuringiensis* var. *israelensis* de Barjac (*B.t.i.*).

Consequently, as a proactive step, the Australian Local Authorities Research Committee funded research into the evaluation of the environmental consequences of mosquito control programs. Therefore, 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 larvivorous fish *Pseudomugil signifer* Kner was selected for laboratory bioassay because it is abundant in freshwater streams, intertidal marshes, and shallow estuarine and wetland habitats in Queensland (Grant 1982). The fish is also a known predator on mosquito larvae (Morton et al. 1988), and is readily cultured in the laboratory.

MATERIALS AND METHODS

Collection, maintenance, and identification of test species: In early 1996, late-juvenile to adult fish 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, Australia). This collection site had not been treated with pesticides since 1994. 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, fish were transferred into 24 × 22 × 46-cm aquaria containing aerated water, and held for a 3- to 4-day period. Recently hatched *Artemia salina* nauplii (Marine laboratory, Hayward, CA) and Wardley's Goldfish Food (Wardley Corporation, Seaucus, NJ) were provided as food. *Pseudomugil signifer* was identified according to the description in Grant (1982).

Test animals: To minimize variability of response to test material, adult fish of uniform length were tested. Fork length (FL) measurements of 20 (10 male and 10 female) freshly killed individuals were taken with Vernier callipers and recorded to the nearest 0.05 mm. Only individuals that were

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

Active ingredient	EFC[1]	LC_{50}[2]	Abiotic characteristics (mean ± SD)				
			Salinity (g/liter)	pH	DO[3] (g/liter)	Turbidity (NTUs)[4]	Temperature (°C)
Temephos (ppm)	0.06	0.594 (0.577, 0.611)	29.0 (±0)	8.1 (±0)	3.6 (±0.3)	0 (±0)	25.0 (±0)
Pirimiphos-methyl (ppm)	0.30	0.091 (0.070, 0.109)	27.0 (±0)	7.3 (±0)	3.7 (±0.5)	0 (±0)	25.0 (±0)
B.t.i. (ITU)	1.279×10^9	6.1×10^{11} (6.03×10^{11}, 6.17×10^{11})	33.5 (±0)	8.6 (±0)	2.2 (±0.2)	0 (±0)	25.0 (±0)
s-Methoprene (ppm)	0.008	>4[5]	39.0 (±0)	7.9 (±0)	2.5 (±0.1)	0 (±0)	25.0 (±0)
Pyriproxyfen (ppm)	0.008	0.845 (0.760,0.971)	31.0 (±0)	7.5 (±0)	3.2 (±0.3)	0 (±0)	25.0 (±0)

[1] EFC, estimated field concentration of active ingredient for 15-cm-deep pools.
[2] LC_{50}, median lethal concentration. Values in parentheses are the 95% lower and upper confidence limits.
[3] DO, dissolved oxygen.
[4] NTUs, nephelometric turbidity units.
[5] No mortality was recorded at 500 times the EFC. Accordingly, the LC_{50} value is greater than 4 ppm.

active after the 3- to 4-day period were used in trials.

Pesticides evaluated: To evaluate the effects of pesticides utilized in field applications, we tested Abate 100E® (AI: 10% temephos applied at 0.1 kg AI/ha, Cyanamid Australia Pty. Ltd., Baulkham Hills, New South Wales, Australia), Actellic® (AI: 90% pirimiphos-methyl applied at 500 ml/ha, ICI Crop Care Pty. Ltd., Melbourne, Victoria, Australia), VectoBac12AS® (AI: 1,200 ITU/mg *B.t.i.* applied at 1.279×10^9 ITU/liter/ha, Hoechst Schering AgrEvo Pty. Ltd., Pennant Hills, New South Wales, Australia), Altosid Liquid Larvicide® (AI: 20% *s*-methoprene applied at 0.06 kg AI/ha, Sandoz Ltd., Dallas, TX), and Sumilarv® (AI: 2% pyriproxyfen applied at 0.06 kg AI/ha, Hoechst Schering AgrEvo). Based on the application rate and the percentage of AI, the estimated field concentration (EFC) in a 15-cm-deep pool was calculated for each pesticide.

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 and fish. 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. Habitat water was used in all the assays as a means of reducing the physiologic stresses associated with translocation to a foreign aquatic environment. 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 control containers holding 20 test specimens each in habitat water without pesticide were used for each bioassay. Initially, the fish were exposed to the EFC for each pesticide. Based on these tests, a range of concentrations that straddled the effective range were tested. 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, fish were not fed for 24 h prior to, or during testing. Because abiotic factors can affect the toxicity of a substance (Cooney 1995), salinity (mg/liter), pH, water temperature (°C), and turbidity (nepholometric turbidity units [NTUs]) 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 taken as an indicator of a 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 5 pesticide doses. To avoid infinite logarithmically transformed values, zero concentrations were ana-

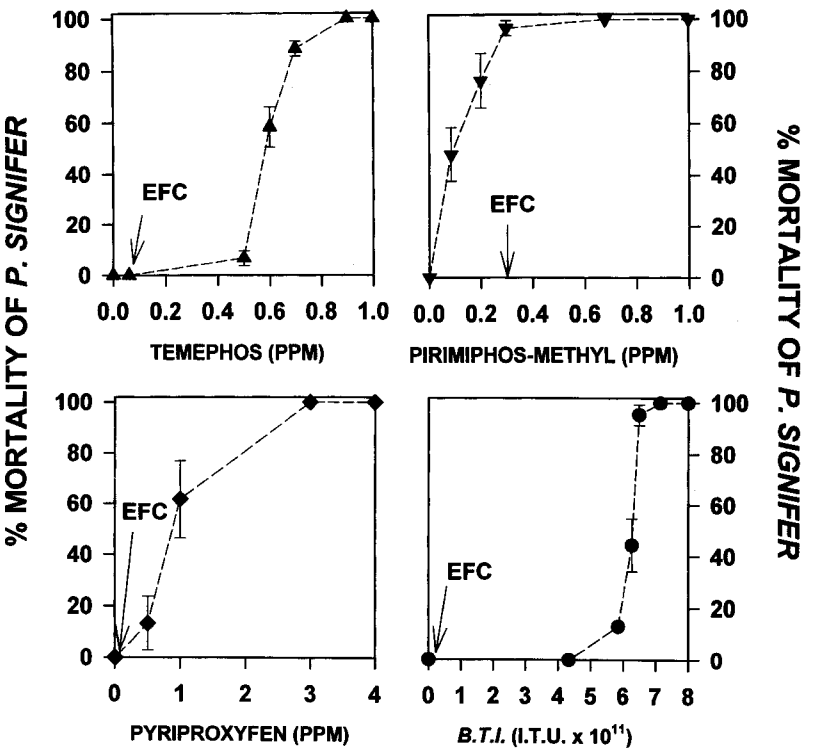

Fig. 1. Concentration–response curves for the fish *Pseudomugil signifer* exposed to temephos, pirimiphos-methyl, pyriproxyfen, and *Bacillus thuringiensis* var. *israelensis* for 96 h. EFC, estimated field concentration.

lyzed 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 27.1 ± 2.1 mm FL was measured for *P. signifer*. The EFC of active ingredient in 15-cm-deep pools for the pesticides evaluated ranged from 0.008 ppb for *s*-methoprene and pyriproxyfen to 0.3 ppm for pirimiphos-methyl (Table 1). Pirimiphos-methyl was the most toxic compound tested against *P. signifer*, with an LC_{50} value of 0.091 ppm (Table 1 and Fig. 1). The LC_{50} value of 0.091 ppm was about one third of the EFC. Temephos, the other organophosphorus compound evaluated, had an LC_{50} value of 0.594 ppm. This represented 9.9 times the EFC. *s*-Methoprene was the least toxic compound, with no mortality suffered at 500 times the EFC. With an LC_{50} value of 0.854 ppm (106 times the EFC), pyriproxyfen was more toxic to *P. signifer* than was *s*-methoprene. VectoBac12AS exhibited low acute toxicity to *P. signifer*, with an LC_{50} value that was 477 times EFC. No control mortality was recorded.

DISCUSSION

In addition to the food chain importance of nontarget species, protection of naturally occurring predators of mosquitoes will reduce the cost of pesticide applications. Accordingly, for routine mosquito control, we only recommend the use of pesticides with proven low toxicity to nontarget species.

In terms of acute toxicity, the organophosphorus compound pirimiphos-methyl was the most lethal of the 5 compounds tested. Exposure to this pesticide produced high levels of mortality at concentrations below those recommended for the control of mosquito larvae. We have been unable to find results of other tests of this compound on estuarine organisms, nor have we at this stage tested it on a wide range of species, but clearly care should be exercised in its use.

Although crustaceans appear to be more susceptible to temephos than is *P. signifer* (Mortimer and Chapman 1995, Brown et al. 1996), care should be exercised with this product. With an LC_{50} value of 0.59 ppm, overdosing may induce hyperventilation

and bradycardia (Gehrke 1988). Also, temephos has been shown to concentrate at the surface of aquatic habitats, and to bioaccumulate in fish (Pierce et al. 1996). Accordingly, *P. signifer* is particularly at risk because the fish is a surface feeder.

Pyriproxyfen has been found to be toxic to *Leander tenuicornis,* a cohabiting estuarine shrimp, at concentrations slightly in excess of those recommended for mosquito control (Brown et al. 1996). In contrast, *P. signifer* tolerated pyriproxyfen at levels far in excess of the EFC. However, with respect to nontarget safety, these studies have shown that *s*-methoprene is the safer of the two IGRs.

Results from these acute static tests indicate that *s*-methoprene and *B.t.i.* can be safely applied in situations where *P. signifer* occurs. However, there are other considerations to be explored, both biological and environmental, which may affect concentrations of treatment compounds in field situations. These include tidal flushing and dispersal (Pierce et al. 1996), adsorption to organic matter (Cooney 1995), the size and life-history stages of the nontarget species (Mian and Mulla 1992), and synergistic effects with other pollutants (Elcin 1995; Rand 1995).

In our assays we used collection site water for transport, maintenance, and experimentation. This water may have contained unknown pesticides and metals. Fortunately, the excellent control survival recorded during our evaluations strongly indicated that the concentration–response relationships we determined were a direct result of exposure of *P. signifer* to the 5 pesticides under evaluation.

Also, in view of the fact that abiotic factors can influence the toxicity of a substance (Cooney 1995), we believe that it is important to highlight the fact that the *s*-methoprene bioassays were conducted in water with a salinity of 39 mg/liter (Fig. 1). This contrasts with the other evaluations, which occurred in water with salinities ranging from 27 to 33.5 mg/liter. Despite this difference, we have no hesitation in endorsing use of *s*-methoprene, because exposure to 500 times the EFC had no effect on survival of *P. signifer.*

However, it is important to emphasise that conclusions drawn from laboratory tests tend to be conservative. Acute lethal tests cannot indicate likely sublethal influences on behavior, fecundity, growth, and survival of all life-history stages, which may be equally deleterious ecologically. Clearly, further study is required to define these less obvious effects.

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