

Toxicity of δ -phenothrin and resmethrin to non-target insects

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Abstract

BACKGROUND: The susceptibility of adult house cricket, *Acheta domesticus* (L.), adult convergent lady beetle, *Hippodamia convergens* (Guérin-Méneville), and larval fall armyworm, *Spodoptera frugiperda* (JE Smith), to resmethrin and δ -phenothrin synergized with piperonyl butoxide (PBO) was evaluated in a laboratory bioassay procedure.

RESULTS: The 1 day LC₅₀ values for resmethrin + PBO were 23.2, 32.08 and 307.18 ng cm⁻² for *A. domesticus*, *H. convergens* and *S. frugiperda* respectively. The 1 day LC₅₀ values for δ -phenothrin + PBO were 26.9, 74.91 and 228.57 ng cm⁻² for *A. domesticus*, *H. convergens* and *S. frugiperda* respectively. The regression relationship between species mortality and concentration explained 51–81% of the variation for resmethrin + PBO and 72–97% of the variation for δ -phenothrin + PBO. The LC₅₀ values decreased with time for these insecticides for all surrogate species. In terms of sensitivities among the insects to resmethrin + PBO and δ -phenothrin + PBO, *A. domesticus* was most sensitive, followed by *H. convergens* and then *S. frugiperda*.

CONCLUSION: The results indicate that resmethrin + PBO was generally more toxic than δ -phenothrin + PBO. Based on the results, *A. domesticus* seems to be a good surrogate species for estimating potential non-target terrestrial insect impacts from exposure to pyrethroids used in public health applications.

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Keywords: risk assessment; ecotoxicology; house cricket; convergent lady beetle; fall armyworm; *Acheta domesticus*; *Hippodamia convergens*; *Spodoptera frugiperda*; insecticide toxicity

1 INTRODUCTION

Pyrethroids are broad-spectrum insecticides with rapid knockdown activity and are among the most effective groups of insecticides.¹ In public health, δ -phenothrin [3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate] and resmethrin [5-benzyl-3-furylmethyl (1*RS*)-*cis-trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)-cyclopropanecarboxylate] are synthetic pyrethroids used against mosquitoes, human lice and flying and crawling insects in homes and recreational areas.^{2–6} With the increased prevalence of mosquito-borne pathogens such as West Nile virus in recent years, pyrethroids such as permethrin, resmethrin and δ -phenothrin are being used more widely to manage adult mosquitoes.^{7,8}

Associated with the increased use of permethrin, resmethrin and δ -phenothrin are public concerns about the environmental effects of their usage. This is especially true for adult mosquito management using these insecticides because, although the application rates are very low, the application method, ultralow-volume (ULV) spray, essentially causes the insecticides to drift over relatively large areas. Most pyrethroids are toxic to both target and non-target insects.¹ However, most studies of the impact of pyrethroids have been limited to aquatic invertebrates. Data on resmethrin and δ -phenothrin toxicity for non-target terrestrial invertebrates are limited to honey bees.^{3,4}

Risk assessment has been used to quantify risks from mosquito management tactics.^{9–12} In pesticide risk assessment, dose–response relationships for surrogate non-target species are determined from bioassay studies and are used to compare toxic

doses to estimated or actual environmental exposures.^{13,14} This is often accomplished using the risk quotient (RQ) method, whereby the estimated environmental concentration (EEC) is compared with a toxic endpoint (e.g. LC₅₀ or no-effect concentration).¹⁴

Ecological risk assessments of pyrethroids such as resmethrin and δ -phenothrin are limited because of a lack of toxicity data for non-target terrestrial invertebrates. Therefore, the objective of this study was to estimate LC₅₀ values for resmethrin and δ -phenothrin against three non-target, surrogate insect species.

2 MATERIALS AND METHODS

2.1 Insecticides

Technical-grade resmethrin (94.3% purity), δ -phenothrin (94.9% purity), and a synergist, piperonyl butoxide (PBO) (98.2% purity), were obtained from Sigma-Aldrich (St Louis, MO). Stock solutions for δ -phenothrin, resmethrin and PBO were prepared for each pesticide before each experiment by dissolving the insecticides and PBO in acetone, and serial dilutions were made by volumetric pipetting. Concentrations were expressed on a weight/volume basis.

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Table 1. Insecticide treatments and concentrations used

Species	Treatment	Concentration (ng cm ⁻²) ^a										
		0X	0.25X	0.30X	0.35X	0.50X	0.75X	1X	2X	3X	5X	10X
<i>Acheta domesticus</i>	Resmethrin ^b	0.00	19.75	23.7	27.65	39.5	59.24	78.99				
	δ -Phenothrin ^c	0.00	10.11	12.13	14.15	20.21	30.32	40.43				
<i>Hippodamia convergens</i>	Resmethrin	0.00	19.75	23.7	27.65	39.5	59.24	78.99				
	δ -Phenothrin	0.00	10.11	12.13	14.15	20.21	30.32	40.43	80.85			
<i>Spodoptera frugiperda</i>	Resmethrin	0.00	19.75	23.7	27.65	39.5	59.24	78.99	157.97	236.96	394.93	789.87
	δ -Phenothrin	0.00	10.11	12.13	14.15	20.21	30.32	40.43	80.85	121.28	202.13	404.26

^a Concentration: 0X, control (acetone); 0.25X, 0.30X, 0.35X, 0.50X, 0.75X, 1X, 2X, 3X, 5X and 10X the maximum field application rate.

^b PBO concentrations for resmethrin were 0X, control (acetone); 59.24 ng cm⁻² (0.25X), 70.79 ng cm⁻² (0.30X), 82.59 ng cm⁻² (0.35X), 117.98 ng cm⁻² (0.50X), 176.98 ng cm⁻² (0.75X), 235.97 ng cm⁻² (1X), 471.93 ng cm⁻² (2X), 707.90 ng cm⁻² (3X), 1179.83 ng cm⁻² (5X), and 2359.66 ng cm⁻² (10X).

^c PBO concentrations were the same as those of δ -phenothrin.

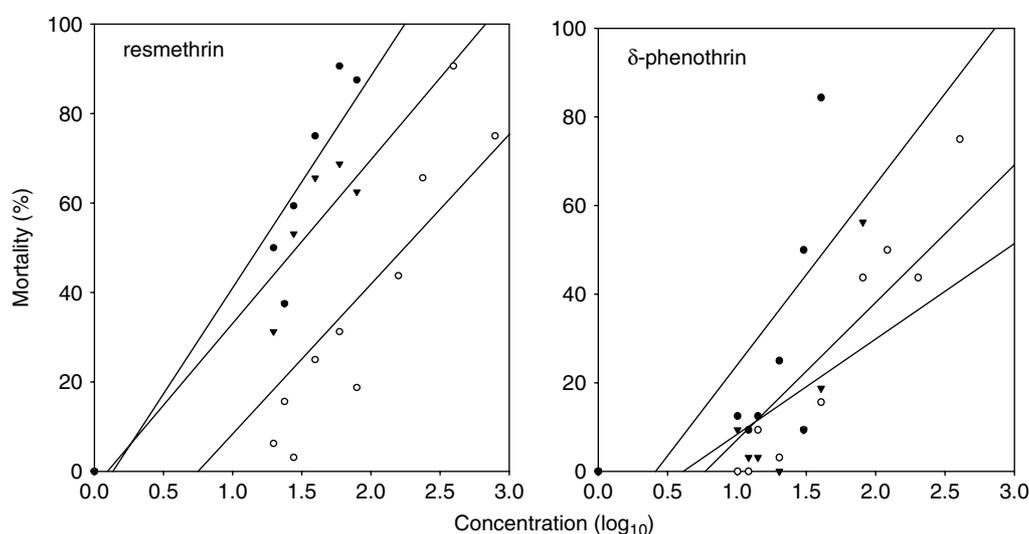


Figure 1. Percentage mortality of *Acheta domesticus* (●), *Spodoptera frugiperda* larvae (○) and *Hippodamia convergens* (▼) versus log concentration of resmethrin and δ -phenothrin at day 1.

Using serial dilution preparations from the stock solutions, a preliminary experiment was conducted to determine the range of insecticide concentrations to use. This was done by exposing the insects to resmethrin + PBO and δ -phenothrin + PBO at decreasing concentrations above and below the field application rate until mortality rates greater than 0 and lower than 100% were observed.

2.2 Insects

Three taxa of terrestrial insects were chosen for bioassay: house cricket, *Acheta domesticus* (L.), convergent lady beetle, *Hippodamia convergens* (Guérin-Méneville) and fall armyworm, *Spodoptera frugiperda* (JE Smith). *Acheta domesticus* adults (mean dry weight = 147.7 mg; SD = 35.5 mg) were chosen because of ease of handling and rearing and known susceptibility to many insecticides.¹⁵ *Hippodamia convergens* adults (mean dry weight = 10.4 mg; SD = 9.6 mg) were chosen because of ease of handling and predator trophic level. *Spodoptera frugiperda* larvae were chosen because of ease of handling and rearing and immature life stage. *Acheta domesticus* adults were purchased from Premium Crickets (Thomson, GA), *H. convergens* adults were

provided by Planet Natural (Bozeman, MT) and *S. frugiperda* larvae were purchased in the third stage from Benzon Research (Carlisle, PA).

2.3 Bioassays

The LC₅₀ was determined under laboratory conditions. For the establishment of the concentration–mortality relationships, insects were exposed to 7, 8 and 11 concentrations, depending on experiment and species (0, 0.25, 0.30, 0.35, 0.50, 0.75, 1, 2, 3, 5 and 10 times the maximum field application rates for each insecticide) (Table 1). 'Field application rate' means the maximum rate in g ha⁻¹. The field rates for resmethrin and δ -phenothrin are 7.85 g ha⁻¹ and 4.03 g ha⁻¹ respectively.

Acetone solutions of resmethrin + PBO and δ -phenothrin + PBO were applied in glass vials (length 4.5 cm, diameter 2.5 cm, volume 20 cm³; Thermo Fisher Scientific Inc., Waltham, MA). Pure acetone was used as the control. A quantity of 1 mL of the insecticide dilution (resmethrin or δ -phenothrin) as well as PBO was dispensed with a micropipette into the vials. The vials were then placed on hot dog rollers (model HDR-565; The Helman Group, Ltd, Oxnard, CA) and rotated mechanically to coat the vials uniformly until they

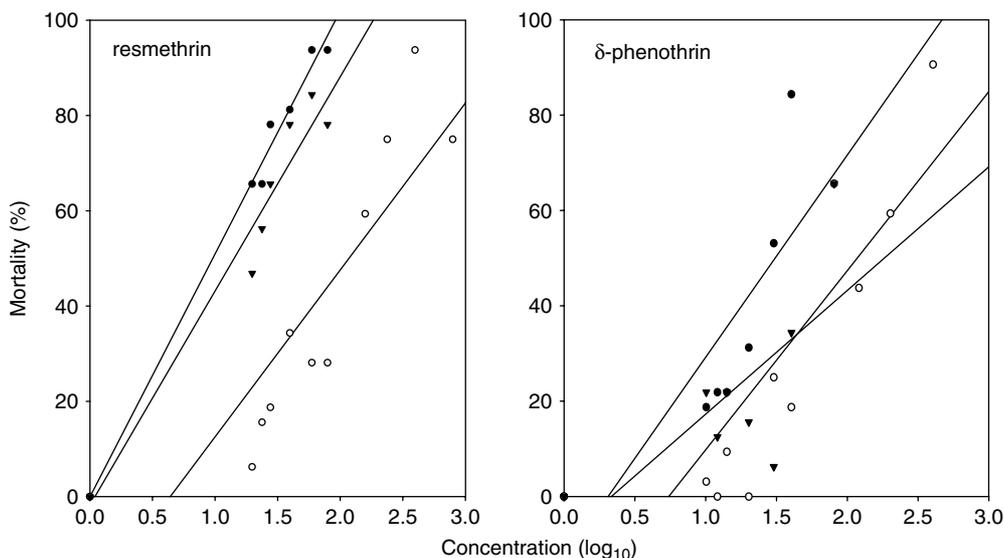


Figure 2. Percentage mortality of *Acheta domestica* (●), *Spodoptera frugiperda* larvae (○) and *Hippodamia convergens* (▼) versus log concentration of resmethrin and δ -phenothrin at day 2.

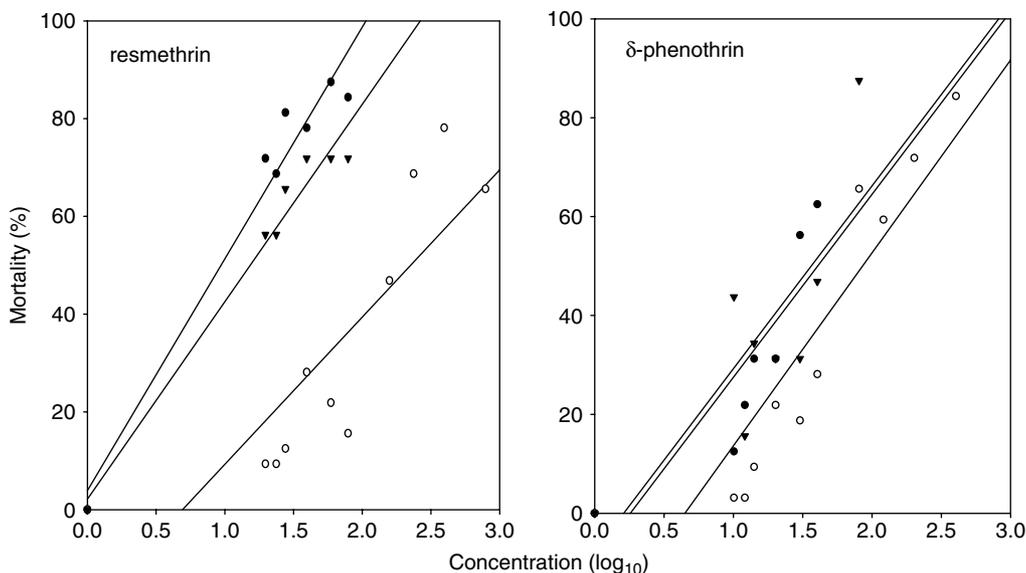


Figure 3. Percentage mortality of *Acheta domestica* (●), *Spodoptera frugiperda* larvae (○) and *Hippodamia convergens* (▼) versus log concentration of resmethrin and δ -phenothrin at day 3.

were dried. One insect was placed in each vial and covered with a perforated cap. Four experimental replicates were used for each concentration of each insecticide. For *S. frugiperda*, the caps were lined with a fine-mesh nylon fabric to prevent escape and to allow air circulation in the vials.

Treated vials were placed on large plastic trays and left on the laboratory bench at $24 \pm 0.5^\circ\text{C}$ with a photoperiod of 16:8 h light:dark. Insect mortalities were assessed and recorded daily for 3 days. The insects were observed for 3 days to account for delayed mortality and exposures over time to potential residues after a single application scenario. Because 3 days represents a considerable time for the insects in the vials, the 1 day mortality data are most likely the most informative. Insects that did not move when prodded with forceps were considered dead. Abbott's formula,¹⁶ as defined by Perry *et al.*¹⁷ and Antwi *et al.*,¹⁸ was used

to correct for control mortality, in which insects were exposed to vials treated with acetone only. Control mortalities were less than 5%.

2.4 Data analysis

The data were analyzed with SAS 9.1.¹⁹ The LC_{50} values were determined with PROC PROBIT, mortality was regressed on concentration using PROC REG¹⁹ and graphs of percentage mortality against \log_{10} concentration were plotted with Sigma Plot 8.0 (SPSS Inc., Chicago, IL). However, for *S. frugiperda* larvae, the poor fit of the models was accounted for by multiplying the variances by a heterogeneity factor ($\chi^2/k - 2$), where k is the number of concentrations, to account for extrabinomial variations due to genetic and environmental influences that caused poor fit.^{20–23} Differences in LC_{50} values among the insects and between

Table 2. Relationship between mortality and concentrations of resmethrin and δ -phenothrin with piperonyl butoxide

Species	Treatment	Day	Regression model ^a	F	R ²	P
<i>Acheta domesticus</i>	Resmethrin	1	$Y = 18.52 + 1.09X$	21.76	0.8131	0.0055
<i>Acheta domesticus</i>	Resmethrin	2	$Y = 32.73 + 1X$	10.21	0.6712	0.0241
<i>Acheta domesticus</i>	Resmethrin	3	$Y = 38.03 + 0.83X$	5.28	0.5135	0.07
<i>Hippodamia convergens</i>	Resmethrin	1	$Y = 17.94 + 0.78X$	11.36	0.6943	0.0199
<i>Hippodamia convergens</i>	Resmethrin	2	$Y = 26.37 + 0.9X$	10.4	0.6752	0.0234
<i>Hippodamia convergens</i>	Resmethrin	3	$Y = 30.44 + 0.73X$	6.2	0.5534	0.0552
<i>Spodoptera frugiperda</i> larvae	Resmethrin	1	$Y = 16.58 + 0.11X$	17.83	0.6646	0.0022
<i>Spodoptera frugiperda</i> larvae	Resmethrin	2	$Y = 22.73 + 0.1X$	12.98	0.5906	0.0057
<i>Spodoptera frugiperda</i> larvae	Resmethrin	3	$Y = 17.35 + 0.09X$	14.09	0.6102	0.0045
<i>Acheta domesticus</i>	δ -Phenothrin	1	$Y = -10.94 + 2.12X$	71.08	0.9343	0.0004
<i>Acheta domesticus</i>	δ -Phenothrin	2	$Y = -3.70 + 2.02X$	181.03	0.9731	<0.0001
<i>Acheta domesticus</i>	δ -Phenothrin	3	$Y = 1.31 + 1.62X$	103.55	0.9539	0.0002
<i>Hippodamia convergens</i>	δ -Phenothrin	1	$Y = -5.69 + 0.7X$	52.96	0.8982	0.0003
<i>Hippodamia convergens</i>	δ -Phenothrin	2	$Y = 3.61 + 0.72X$	23.64	0.7976	0.0028
<i>Hippodamia convergens</i>	δ -Phenothrin	3	$Y = 12.76 + 0.91X$	25.57	0.81	0.0023
<i>Spodoptera frugiperda</i> larvae	δ -Phenothrin	1	$Y = 6.43 + 0.19X$	40.4	0.8178	0.0001
<i>Spodoptera frugiperda</i> larvae	δ -Phenothrin	2	$Y = 9.28 + 0.23X$	31.81	0.7795	0.0003
<i>Spodoptera frugiperda</i> larvae	δ -Phenothrin	3	$Y = 14.79 + 0.22X$	23.7	0.7247	0.0009

^a Y = mortality (%); X = concentration (ng cm⁻²).

Table 3. Lethal concentrations and risk quotients for *Acheta domesticus* treated with resmethrin and δ -phenothrin with piperonyl butoxide

Treatment	Day	LC ₅₀ (ng cm ⁻²)	CI (95%)	P > χ^2	Risk quotient (EEC/LC ₅₀) ^a
Resmethrin ^b	1	23.2	17.34–25.41	0.3429	3.41
Resmethrin	2	13.39	5.03–18.98	0.9104	5.9
Resmethrin	3	4.7	ND ^c	0.8362	16.81
δ -Phenothrin ^d	1	26.9	23.51–32.05	0.2008	1.5
δ -Phenothrin	2	24.37	20.85–29.97	0.2957	1.66
δ -Phenothrin	3	28.14	22.97–39.3	0.8045	1.44

^a EEC = maximum field rate.
^b Resmethrin commercial maximum field rate = 78.99 ng cm⁻² (0.007 lb acre⁻¹).
^c ND, no data as confidence interval could not be determined by statistical analysis.
^d δ -Phenothrin commercial maximum field rate = 40.43 ng cm⁻² (0.0036 lb acre⁻¹).

Table 4. Lethal concentrations and risk quotients for *Hippodamia convergens* treated with resmethrin and δ -phenothrin with piperonyl butoxide

Treatment	Day	LC ₅₀ (ng cm ⁻²)	CI (95%)	P > χ^2	Risk quotient (EEC/LC ₅₀) ^a
Resmethrin ^b	1	32.08	20.76–43.65	0.3542	2.46
Resmethrin	2	17.85	7.11–24.73	0.5472	4.43
Resmethrin	3	10.95	ND ^c	0.9206	7.21
δ -Phenothrin ^d	1	74.91	64.95–90.14	0.3498	0.54
δ -Phenothrin	2	56.02	45.71–73.85	0.1227	0.72
δ -Phenothrin	3	28.29	15.45–52.38	0.0114	1.43

^a EEC = maximum field rate.
^b Resmethrin commercial maximum field rate = 78.99 ng cm⁻² (0.007 lb acre⁻¹).
^c ND, no data as confidence interval could not be determined by statistical analysis.
^d δ -Phenothrin commercial maximum field rate = 40.43 ng cm⁻² (0.0036 lb acre⁻²).

the treatments were determined by comparison of the 95% confidence limits.

3 RESULTS

The results of residual or contact bioassays for the insects are shown in Figs 1, 2 and 3. The lethal concentrations are also presented in Tables 3, 4 and 5. There was generally a good fit to the model assumptions. Table 2 shows the regression relationship between mortality of the insects (*Acheta domesticus*, *Hippodamia convergens*, and *Spodoptera frugiperda* larvae) and resmethrin + PBO and δ -phenothrin + PBO concentrations. (Hereafter, 'resmethrin + PBO' and ' δ -phenothrin + PBO' will be referred to as 'resmethrin' and ' δ -phenothrin' respectively.) The relationships were significant. For resmethrin, the models explained 51.4–81.3% of the

total response variation for *A. domesticus*, 55.3–69.4% for *H. convergens* and 59.1–66.5% for *S. frugiperda* larvae for days 1 to 3 (Table 2). For δ -phenothrin, the models explained 93.4–97.3% for *A. domesticus*, 79.8–89.8% for *H. convergens* and 72.5–81.8% for *S. frugiperda* larvae for days 1 to 3 (Table 2).

For resmethrin, the slopes varied from 0.83 to 1.09 for *A. domesticus*, from 0.73 to 0.9 for *H. convergens* and from 0.09 to 0.11 for *S. frugiperda* larvae (Table 2). For δ -phenothrin, the slopes ranged from 1.62 to 2.12 for *A. domesticus*, from 0.7 to 0.91 for *H. convergens* and from 0.19 to 0.23 for *S. frugiperda* larvae for days 1 to 3 (Table 2).

In terms of sensitivities among the insects to resmethrin and δ -phenothrin, *A. domesticus* were most sensitive, followed by *H. convergens* and then *S. frugiperda* larvae. *Acheta domesticus* was the

Table 5. Lethal concentrations and risk quotients for *Spodoptera frugiperda* larvae treated with resmethrin and δ -phenothrin with piperonyl butoxide

Treatment	Day	LC ₅₀ (ng cm ⁻²)	CI (95%)	P > χ^2	Risk quotient (EEC/LC ₅₀) ^a
Resmethrin ^b	1	307.18	169.62–664.63	<0.0001	0.26
Resmethrin	2	256.67	99.77–684.57	<0.0001	0.31
Resmethrin	3	361.65	203.98–862.8	<0.0001	0.22
δ -Phenothrin ^c	1	228.57	158.83–394.3	<0.0001	0.18
δ -Phenothrin	2	165.02	107.55–309.14	<0.0001	0.25
δ -Phenothrin	3	151.94	91.99–294.3	<0.0001	0.27

^a EEC = maximum field rate.

^b Resmethrin commercial maximum field rate = 78.99 ng cm⁻² (0.007 lb acre⁻¹).

^c δ -Phenothrin commercial maximum field rate = 40.43 ng cm⁻² (0.0036 lb acre⁻¹).

most sensitive species for resmethrin (LC₅₀ = 4.7–23.2 ng cm⁻²) and for δ -phenothrin (LC₅₀ = 24.37–28.14 ng cm⁻²) (Table 3). *Hippodamia convergens* LC₅₀ values were 10.95–32.08 ng cm⁻² for resmethrin and 28.29–74.91 ng cm⁻² for δ -phenothrin (Table 4). *Spodoptera frugiperda* larvae were the least sensitive species for resmethrin (LC₅₀ = 256.67–361.65 ng cm⁻²) and for δ -phenothrin (LC₅₀ = 151.94–228.57 ng cm⁻²) (Table 5).

For *A. domesticus*, the lethal concentrations decreased with time, except at day 3 for δ -phenothrin. The LC₅₀ values were 3.41–16.81-fold less than the field rate for resmethrin and 1.44–1.66-fold less than the field rate for δ -phenothrin for days 1 to 3 (Table 3). For *H. convergens*, lethal concentrations decreased with time for resmethrin (32.08–10.95 ng cm⁻²) and for δ -phenothrin (74.91–28.29 ng cm⁻²). The LC₅₀ values were 2.46–7.21-fold less than the field rate for resmethrin and 0.54–1.43-fold less than the field rate for δ -phenothrin for days 1 to 3 (Table 4). Except at day 3 for resmethrin, the LC₅₀ for *S. frugiperda* larvae decreased with time (307.18–256.67 ng cm⁻² for resmethrin and 228.57–151.94 ng cm⁻² for δ -phenothrin) (Table 5). The LC₅₀ values were 0.22–0.31-fold less than the field rate for resmethrin and 0.18–0.27-fold less than the field rate for δ -phenothrin for days 1 to 3 (Table 5).

4 DISCUSSION AND CONCLUSIONS

The use of surrogate species is an established testing strategy to assess the potential impact of a chemical on species residing in the habitat of concern. The results of this study indicate that resmethrin and δ -phenothrin are inherently more toxic to *Acheta domesticus* than to *Hippodamia convergens* or *Spodoptera frugiperda* larvae.

For each active ingredient there was a good relationship between the observed mortality of the insects and the concentrations used. The regression relationship explained 51–97% of the variation in the models for all treatments and insect species tested. The slope of the regression relationship line indicates how fast the insects responded with increasing concentration. The slopes were generally higher for *A. domesticus*, varying between 0.83 and 2.12 for both resmethrin and δ -phenothrin. *Spodoptera frugiperda* larvae had the lowest slopes of about 0.09–0.23 for resmethrin and δ -phenothrin.

The results demonstrate that non-target surrogate insect species representing three orders and families vary in their sensitivity to resmethrin and δ -phenothrin. Comparison of the LC₅₀ values revealed that *S. frugiperda* larvae were least susceptible to resmethrin and δ -phenothrin. The house cricket, *Acheta domesticus*, was the most susceptible species, and, based on the results, this seems to be a good surrogate species for estimating

potential non-target terrestrial insect impacts from exposure to pyrethroids used in public health applications.

Levels of concern (LOC) are tools that policy makers and regulatory agencies use to assess the acceptability of risks.¹⁴ The ratio between the estimated environmental concentration (EEC) and LC₅₀, termed the risk quotient (RQ), gives an estimate of the risk. The calculated RQ is compared with the respective RQ LOC to determine if there is a need for regulatory action.¹⁴ The USEPA typically uses an acute RQ LOC of ≥ 0.5 for terrestrial animals (i.e. the estimated exposure is 50% of the LC₅₀). If it is assumed that the EECs are equivalent to the field application rates (assuming 100% even deposition of insecticide over 1 ha), daily RQs for *A. domesticus* and *H. convergens* were > 0.5 for resmethrin and δ -phenothrin. RQs for *S. frugiperda* larvae did not exceed 0.5 for resmethrin or δ -phenothrin.

These results suggest that mortality risks would be greater for resmethrin than for δ -phenothrin. Risk quotients for some non-target terrestrial insects may exceed RQ LOCs. However, it is highly unlikely that pyrethroid deposition on terrestrial surfaces after ULV applications would be 100% of application rate. Small droplets produced from ULV sprays are distributed by wind over a wide area, and observed deposition rates have been low.^{15,24} Indeed, the weight of evidence suggests that surface deposition ranges from approximately 1 to 10%.^{12,24,25} If terrestrial deposition were 10% of the application rate, then the EECs from the maximum ULV application rates for the insecticides in this study would result in RQs that exceeded 0.5 only for *A. domesticus* and *H. convergens* exposed to resmethrin on days 2 and 3. No other RQ LOCs would be exceeded.

Toxicities as determined by LC₅₀ values were lower on day 1 for resmethrin and δ -phenothrin for all surrogate species compared with those on days 2 and 3. The toxicities were greater on the second and third days primarily because the insects had been exposed continuously to the insecticides in the vials for 72 h. In contrast to the exposure in the vial, exposure in the field most likely is much different. Knepper *et al.*²⁴ observed very low persistence of malathion and permethrin, with only trace levels after 36 h. Additionally, the adsorption of resmethrin and δ -phenothrin to soil particles and vegetation in the terrestrial environment may reduce their bioavailability, further limiting their effects on non-target insects.

In a deterministic, reasonable worst-case risk assessment, Davis *et al.*¹⁰ concluded that acute and chronic risks to ecological receptors, including terrestrial insects, from ULV insecticides used for mosquito management most likely are low. However, toxicity data for non-target insects were not available at the time of their

study. The present results provide LC₅₀ values for surrogate, non-target terrestrial insects and can be compared with actual and estimated environmental insecticide concentrations to estimate risks. Given the LC₅₀ values that were estimated and what is currently known about the fate of resmethrin and δ -phenothrin after ULV application, the present results may be used to support the conclusions of Davis *et al.*¹⁰

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