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Toxicity and risk of permethrin and naled to non-target insects after adult mosquito management

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Abstract We derived laboratory LC50 values, assessed non-target insect risks, and conducted a field bioassay for ultra-low-volume (ULV) aerosol applications of insecticides used to manage adult mosquitoes. The house cricket, *Acheta domesticus* (L.), was used as an indicator species for medium- to large-bodied ground dwelling insects. The 24-h LC_{50} values for Permanone® (formulated product of permethrin), Permanone + piperonyl butoxide (PBO), technical grade permethrin, and technical grade permethrin + PBO ranged from 0.052 to 0.9 μg/cm^2. The 24 h LC_{50} for technical grade naled and Trumpet® (formulated product of naled) were 0.038 and 0.44 μg/cm^2, respectively. The synergist ratio was 2.65 for Permanone + PBO and 1.57 for technical grade permethrin + PBO. The toxicity of technical grade permethrin was about 10-fold greater than Permanone. A risk assessment using modeled estimated environmental concentrations resulted in risk quotients (RQ) that exceeded regulatory levels of concern, but when compared to field-derived actual environmental concentrations RQs did not exceed a regulatory level of concern, except in the case of technical grade naled. These results were expected because higher tiered risk assessments using field-verified data generally lead to lower risk estimates. Field bioassays using caged crickets showed no significant mortality for permethrin or naled after a single truck-mounted ULV application. The results of the risk assessment using actual environmental concentrations are supported by the field bioassays and suggest that a single ULV application of synergized or unsynergized permethrin and naled most likely will not result in population impacts on medium- to large-bodied insects.

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Introduction

West Nile virus (WNV), a mosquito-borne arbovirus, has become endemic to North America and disease cases occur throughout the virus transmission season. Since the arrival of WNV, more areas of the country have experienced large-scale insecticide applications. Ultra-low-volume (ULV) aerosol applications of insecticides are used to manage high densities of adult mosquitoes and are the minimum effective volume of insecticide that is used as an outdoor space spray. Currently, adult mosquito management utilizes pyrethroids and pyrethrins ((Z)-(S)-2-methyl-4-oxo-3-(penta-2,4-dienyl)cyclopent-2-enyl (1R,3R)-2,2dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate) synergized with piperonyl butoxide (PBO; 5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole), or organophosphate insecticides (Rose 2001).

The insecticides commonly used for mosquito management are highly toxic to non-target organisms like aquatic and terrestrial invertebrates and aquatic vertebrates, and there have been concerns about the effect of ULV applications on these organisms (Amweg et al. 2006; Davis et al. 2007; Paul and Simonin 2006; Paul et al. 2005; Schleier III et al. 2008; Weston et al. 2006). Pyrethrins, pyrethroids, and organophosphate ULV insecticide sprays have had detrimental effects on non-target organisms like honey bees (Caron 1979; Hester et al. 2001; Pankiw and Jay 1992; Womeldorf et al. 1974; Zhong et al. 2003).

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Aerial applications of ULV malathion (O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate) significantly decreased populations of certain Hymenoptera (Hill et al. 1971). However, the aerial applications did not significantly affect Hemiptera, Coleoptera, and Diptera (excluding Culicidae) populations (Hill et al. 1971).

Truck-mounted applications of ULV permethrin (3-phenoxybenzyl(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) had no significant impact on aquatic macroinvertebrates and *Gambusia affinis* (Baird and Girard) when used above wetlands, but did have a significant impact on small flying insects (Jensen et al. 1999). Flying insect populations, though, recovered within 48 h after the initial application. Aerial ULV applications with pyrethrins and PBO had no significant impact on caged medium- to large-bodied insects within the spray zone, but researchers observed an impact on smaller-bodied insects (Boyce et al. 2007). Davis et al. (2007) found that the exposures to mammals, birds, and aquatic vertebrates and invertebrates most likely would not result in risks that exceed regulatory levels of concern after truck-mounted ULV applications. Davis and Peterson (2008) demonstrated that there was little to no significant impact on aquatic and terrestrial invertebrates after single and multiple applications of permethrin or δ-phenothrin (3-(phenoxy)phenyl] methyl (1R,3R)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate) used for adult mosquito management. Lawler et al. (2008) found that the use of ULV pyrethrins synergized with PBO did not cause significant mortality of the aquatic invertebrates, *Daphnia magna* Straus and *Callibaetis californicus* Banks. However, truck-mounted ULV applications of malathion have been shown to have a significant effect on house crickets, *Acheta domesticus* Linnaeus in a peridomestic setting (Tietze et al. 1996), causing 12.5–48.7% mortality, depending on the location in residential yards.

There have been few studies examining the effects of truck-mounted ULV applications on non-target ground dwelling organisms. In addition, there are few data on non-target arthropods with respect to toxicity and risks associated with insecticides used for adult mosquito management. Therefore, the objectives of our study were to determine the susceptibility of house crickets to permethrin and naled, and perform an ecological risk assessment using the house cricket as a surrogate for medium- to large-bodied ground dwelling arthropods. In addition to the toxicity testing and risk assessment, caged crickets were placed in the field to determine if the insecticide applications caused significant mortality.

Despite the well known approaches in risk assessment of tiers, recursiveness, and refinement (NRC 1983; SETAC 1994), surprisingly few studies have been conducted and published that quantitatively characterize the change in risk from lower to higher tiered assessments for non-target organisms (Giddings et al. 2001; Peterson 2006; Schleier III et al. 2008). Therefore we compared estimated environmental concentrations to actual environmental concentrations to demonstrate the change in risk when higher tiered assessments are performed.

Materials and methods

Laboratory bioassay

Adult house crickets (mean dry wt. = 148.75 mg; SD = 51.71 mg) were obtained from Big Apple Herpetological (Hauppauge, NY, USA) for both the laboratory and field experiments. House crickets were chosen for the lab and field experiments because they are considered to be one of the best indicators of environmental pollution because they are more susceptible than other species of invertebrates (Antwi and Peterson 2009; Bass 1986; Brieger et al. 1992; Harris 1966; Hoffmann et al. 2002; Tietze et al. 1996).

Technical grade permethrin (98% purity) and synergist PBO (98.2% purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA), Permanone® 10% emulsified concentrate (EC) (permethrin) was obtained from Bayer Environmental Science (Research Triangle Park, NC, USA), Trumpet® EC (naled; 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) was obtained from the AMVAC Corporation (Los Angeles, CA, USA), and technical grade naled (98.2% purity) was obtained from Chem Service (West Chester, PA, USA). Gas chromatograph analysis of Permanone and Trumpet showed that the percent active ingredient in the formulations is 10.05 and 78%, respectively. Stock solutions for technical grade permethrin, technical grade permethrin + PBO (1:1 technical grade permethrin/PBO), Permanone, Permanone + PBO (1:1 Permanone/PBO), Trumpet, and technical grade naled were prepared for each experiment in high pressure liquid chromatography acetone (99.7% purity; EMD Chemicals, Gibbstown, NJ, USA). Concentrations of Permanone were based on 0.84 kg/l, the amount of permethrin in the formulation. Concentrations of Trumpet were based on 1.29 kg/l, the amount of naled in the formulation.

The LC_{50} was determined under laboratory conditions using methods similar to those of Snodgrass (1996) and Antwi and Peterson (2009). Acetone solutions of the different active ingredients listed above were applied in 20-ml glass scintillation vials with a total inside surface area of 40.26 cm^2 (Thermo Fisher Scientific Inc., Waltham, MA, USA). A 0.5-ml aliquot of test solution was dispensed into individual glass vials. Acetone was used as the control. Vials were placed on hot dog rollers (model HDR-565, The

Helman Group, Ltd., Oxnard, CA, USA) and rotated mechanically so that the acetone dried and the insecticide was uniformly coated in the vial. One insect was placed in each vial and covered with a perforated cap, without diet. Treated vials were placed on large plastic trays and left on the laboratory bench (21.28 ± 0.04°C, photoperiod of 16:8 [L:D] h). Mortality was assessed at 24 h, and insects that did not move when stimulated with forceps were considered dead. All combinations of permethrin were run at the same time so that differences between the treatments and times could be analyzed.

To establish the concentration-mortality relationships the experiments were performed four times so that dose–response curves could be produced. The experimental design for the permethrin experiments was a complete randomized block with eight vials (individuals) per concentration, 12 concentrations (blocks), and four treatments (technical grade, technical grade + PBO, Permanone, and Permanone + PBO). The experimental design for the naled experiments was a complete randomized block with eight vials (individuals) per concentration, 12 concentrations (blocks), and two treatments (technical grade naled and Trumpet).

To establish the concentration-mortality relationships for Permanone and Permanone mixed with PBO, insects were exposed to 12 concentrations with 32 individuals exposed at each concentration: 0, 0.026, 0.078, 0.13, 0.16, 0.19, 0.26, 0.65, 0.78, 1.04, 1.3, and 2.6 μg/cm^2. To establish the concentration-mortality relationships for technical grade permethrin and technical grade permethrin mixed with PBO, insects were exposed to 12 concentrations with 32 individuals exposed at each concentration: 0, 0.015, 0.031, 0.037, 0.07, 0.093, 0.12, 0.16, 0.19, 0.23, 0.31, and 0.78 μg/cm^2. Concentration-mortality relationships were established for technical grade naled and Trumpet using 12 concentrations with 32 individuals exposed at each concentration: 0, 0.0078, 0.016, 0.0548, 0.078, 0.12, 0.2, 0.24, 0.32, 0.39, 0.79, and 1.59 μg/cm^2.

Data analysis

Abbott's formula was used to correct for control mortality (Abbott 1925; Perry et al. 1998). Synergist ratios were calculated by dividing the unsynergized LC_{50} by the synergized LC_{50} (Casabe et al. 1988). Data were analyzed using Statistical Analysis System 9.1 (SAS Institute, Cary, NC, USA) and dose-mortality regressions were estimated by probit analysis (PROC PROBIT). However, for Permanone the poor fit of the model was accounted for by multiplying the variances by a heterogeneity factor (χ^2/k − 2), where k is the number of concentrations to account for extra-binomial variations that causes poor fit (Hoekstra 1991; Robertson et al. 2007; Williams 1982). Significant differences ($p \leq 0.05$) between permethrin and naled LC_{50} estimates were determined by the LC_{50} ratio test of Wheeler et al. (2006).

Field bioassays

The study occurred in conjunction with the Cascade County Weed and Mosquito Control District outside of Cascade (N47°13.489′, W111°42.040′), and Ulm (N47°25.402′, W111°29.767′), MT, USA during the summers of 2007 and 2008, respectively. In 2007 only Trumpet (formulated product of naled) was applied, while in 2008 both Permanone and Trumpet were applied. Each insecticidal product was applied once per year in late July or early August (Schleier III and Peterson 2010). A truck was equipped with a Bison (VecTec Inc., Orlando, FL, USA) ULV generator. Permanone 10% EC was mixed 1:1 with BVA oil (BVA Inc. Wixom, MI, USA) and was applied at the maximum application rate of 7.85 g active ingredient (ai)/ha with a flow rate of 205 ml/min. Trumpet was applied undiluted at the maximum application rate of 22.42 g ai/ha with a flow rate of 44.36 ml/min. Schleier III and Peterson (2010) provide further information on the applications.

Approximately 15 adults were placed in a 25-cm × 25-cm × 8-cm mesh wire screen cage. One cage of crickets was placed on the ground 25, 50, and 75 m from the spray truck. There were three sample replicates with 200 m buffer zones between replicates. Three cages of crickets were placed in a control area located where no spraying or drift could occur, but were subjected to the same meteorological conditions as the residue samples. Cricket mortality was assessed 2 h after application because of low overnight temperatures. BoxCox transformations were run on mortality to determine the correct transformation. Analysis of variance ($\alpha = 0.05$) was run on mortalities that were transformed by ln(y + 1) to determine differences between crickets in the treated and control areas.

Non-target insect risk assessment

Ecological risk assessment can be described in quantitative terms as a function of toxicity and exposure (NRC 1983; USEPA 1998). The LC_{50} values at 24 h were compared with the actual environmental concentrations (AEC) measured by Schleier III and Peterson (2010) and estimated environmental concentrations (EEC) from the industrial source complex dispersion model version 3 (ISCST3; http://www.epa.gov/scram001/tt22.htm#screen), which has been used to estimate the ground deposition concentrations of insecticides after ULV applications in previous risk assessments (Davis et al. 2007; Macedo et al. 2007;

Peterson et al. 2006; Schleier III et al. 2009a, b) and has been shown to be a sufficiently conservative model for conducting lower tiered risk assessments (Schleier III and Peterson 2010).

Peterson et al. (2006) and Davis et al. (2007) contain more information on the ISCST3 modeling assumptions, which are briefly reviewed here. The assumptions included: (a) permethrin had a 24-h half-life in the air and naled had a 18-h half-life; (b) the insecticides were applied at the maximum application rate as stated on each label; (c) all of the insecticides were susceptible to the same weather conditions using standardized weather data from Salem, Massachusetts, USA, from the year 1988; (d) all spray events occurred at 2100 h; (e) each spray was released at 1.5 m; and (f) deposition concentrations were estimated 25 m from the spray source.

Actual environmental concentrations of permethrin and naled were measured by Schleier III and Peterson (2010). The applications took place in open fields with no vegetation taller than 20 cm, to represent a worst-case assessment of ground deposition. Within each study site, surface residue samples were taken. Three replicates with 200 m buffer zones between replicates were used. The collection of surface residues at ground level were performed using 10 cm × 10 cm (100 cm^2) cotton dosimeters pinned to a piece of cardboard at 25 m from the spray truck and were collected 1 h after application.

The average deposition concentrations 25 m from the spray truck for permethrin estimated by ISCST3 and AECs were 0.18 and 0.0024 $\mu g/cm^2$, respectively. The average deposition concentrations 25 m from the spray truck of naled estimated by ISCST3 and AECs were 0.34 and 0.036 $\mu g/cm^2$, respectively.

We used the risk quotient (RQ) method for estimating risk, which is calculated by dividing the deposition concentration by the LC_{50}. Estimated RQs typically are compared to a RQ level of concern (LOC) which is set by the United States Environmental Protection Agency (USEPA) or another regulatory agency to determine if regulatory action is needed. The RQ LOC used for our assessment was 0.5 (USEPA 1992, 2006). An RQ $\geq$ 0.5 means the estimated exposure is $\geq$50% of the LC_{50}. To assess the change in risk when higher tiered risk assessments are performed we compare the RQ for the AECs and EECs.

Results

Toxicity testing

The LC_{50} values for Permanone, Permanone + PBO, technical grade permethrin, technical grade permethrin + PBO, technical grade naled, and Trumpet are given in Table 1. There was generally a good fit to the model assumptions. Technical grade permethrin alone and synergized was significantly more toxic than the permethrin in Permanone alone and synergized. The addition of PBO did not significantly increase the toxicity of Permanone or technical grade permethrin. The 24 h LC_{50} values for Permanone, Permanone + PBO, technical grade permethrin, and technical grade permethrin + PBO ranged from 0.052 to 0.9 $\mu g/cm^2$ (Table 1). The synergist ratio of Permanone + PBO and technical grade permethrin + PBO was 2.65 and 1.57, respectively. The 24 h LC_{50} for technical grade naled and Trumpet were 0.038 and 0.44 $\mu g/cm^2$, respectively.

Field bioassay

There was no significant difference in mortality between crickets in the control and treated areas for the Permanone ($F = 0.08$, $p = 0.78$) and naled spray events ($F = 1$, $p = 0.33$). During the 2007 naled application, average mortality was 18% in the treated area and 10% in the control area. During the 2008 permethrin application, average mortality was 5% in the treated area and 6% in the control area. During the 2008 naled application, average mortality was 1% in the treated area and 3% in the control area.

Risk assessment

Environmental concentrations estimated by ISCST3 resulted in RQs that exceeded the LOC for Permanone + PBO, technical grade permethrin, technical grade permethrin + PBO, technical grade naled, and Trumpet, but Permanone alone did not exceed the RQ LOC (Table 2). Risk quotients using the EECs exceeded the RQ LOC, which were about 10- to 100-fold greater than the RQs using AECs (Table 2). The RQ for technical grade naled exceeded the RQ LOC when using AECs (Table 2).

Discussion

When examining the risks of insecticides to non-target arthropods, it is important to consider the AECs of insecticides and the toxicity of the formulated products. In their ecological risk assessment, Davis et al. (2007) used the conservative model ISCST3 to estimate concentrations of mosquito insecticides that would be deposited on the ground. The concentrations estimated by ISCST3 were 75 and 9.5 times greater for permethrin and naled than the AECs used in this study, respectively (Schleier III and Peterson 2010). The RQs for synergized Permanone, synergized and unsynergized technical grade permethrin, technical grade naled, and Trumpet exceeded the RQ LOC

Table 1 The 24 h lethal concentrations that kill 50% of a population (LC_{50}) for house crickets treated with Permanone® (formulated product of permethrin), Permanone and piperonyl butoxide (PBO), technical permethrin, technical permethrin and PBO, technical naled, Trumpet® (formulated product of naled)

Treatment	Slope ± SE[a]	LC_{50} (μg/cm^2)	CI (95%)[b]	χ^2	df[c]	$P > \chi^2$
Permanone®	2.73 ± 0.37	0.9	0.71–1.29	62.74	9	<0.0001
Permanone + PBO[d]	4.7 ± 0.51	0.34	0.29–0.4	5.25	8	0.73
Technical permethrin	2.91 ± 0.47	0.082	0.06–0.1	13.35	9	0.15
Technical permethrin + PBO	3.41 ± 0.52	0.052	0.039–0.065	6.15	9	0.72
Technical naled	2.89 ± 0.29	0.038	0.034–0.045	10.74	8	0.23
Trumpet®	3.53 ± 0.54	0.44	0.36–0.56	7.78	5	0.17

[a] Standard error

[b] 95% confidence interval for the LC_{50}

[c] Degrees of freedom

[d] Piperonyl butoxide

Table 2 Risk quotients (deposition concentration/LC_{50}) for the actual environmental concentration (AEC) from Schleier III and Peterson (2010) and estimated environmental concentrations modeled with ISCST3[b] for Permanone® (formulated product of permethrin), Permanone + piperonyl butoxide (PBO), technical grade permethrin, technical grade permethrin + PBO, technical grade naled, and Trumpet® (formulated product of naled) at 24 h

Chemical	AEC	ISCST3
Permanone®	0.0027	0.2
Permanone + PBO[a]	0.0071	0.53
Technical grade permethrin	0.029	2.19
Technical grade permethrin + PBO	0.046	3.46
Technical grade naled	0.95	9
Trumpet®	0.081	0.78

[a] Piperonyl butoxide

[b] The industrial source complex dispersion model version 3

when compared to the EECs of ISCST3. However, when the AECs were used, the RQs for all insecticide combinations were below the RQ LOC, except for technical grade naled.

There have been few studies examining the change in risk as more refined risk assessments are performed. Not surprisingly, our estimates of risks for non-target ground dwelling arthropods using AECs were lower than the risks using EECs. These results are expected because higher tiered risk assessments using field verified data generally lead to lower risk estimates which has been observed for aerial applications of ULV insecticides (Schleier III et al. 2008). Our results were supported by the field bioassay showing no significant difference in mortality between crickets in the control and treated areas for either the Permanone or Trumpet treatments.

Although we used AECs to estimate risks, the RQs most likely still overestimate the risks to non-target ground dwelling insects. For example, to derive the LC_{50} the insecticide was coated on the inside of glass vials so that the house crickets were in constant contact with the insecticide. However, in the field non-target organisms most likely are not in constant contact with the insecticide. In addition, house crickets have been shown to be more sensitive to pyrethroid insecticides than smaller non-target organisms such as adult convergent lady beetles, *Hippodamia convergens* (Guérin-Méneville) (Antwi and Peterson 2009).

The toxicity of technical grade permethrin was about 10-fold greater than Permanone. Previous studies have shown that the *cis*-isomer of permethrin is more toxic than the *trans*-isomer (Alzogaray et al. 1998). However, the increased toxicity of the technical grade cannot be explained by this fact because technical grade permethrin contained 26.4% *cis*-isomer, while the Permanone formulation contained 35%. Stevens et al. (2002) found that technical grade organophosphates have a higher toxicity than the formulated products. Paul et al. (2005) and Coats and O'Donnell-Jeffery (1979) found that technical-grade pyrethroids can be two to nine times more toxic than the formulated product to fish. The increased toxicity of technical grade permethrin could be due to a more rapid penetration through the cuticle because high boiling point oils and emulsifiers like those in Permanone can slow the penetration of pyrethroid insecticides (Coats and O'Donnell-Jeffery 1979; Matsumura 1985; Pree et al. 1996).

The toxicity of permethrin to house crickets is similar to that of adult tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), green stink bugs, *Acrosternum hilare* (Say), and southern green stink bugs, *Nezara viridula* (L.), using methods similar to our study (Snodgrass 1996; Snodgrass et al. 2005). The LC_{50} values for technical grade permethrin + PBO were similar to technical grade resmethrin (5-benzyl-3-furylmethyl (1*RS*,3*RS*;1*RS*,3*SR*)-2,2-dimethyl-3-(2-methylprop-1-enyl) Cyclopropanecarboxylate) and δ-phenothrin synergized with PBO (Antwi and Peterson 2009).

Piperonyl butoxide is commonly used with pyrethroids for mosquito management because it can suppress or delay the onset of resistance to pyrethroids and pyrethrins as well as reduce the concentration needed to have a lethal effect (Farnham 1998; Xu et al. 2005). The results of our study showed a synergist ratio of 1.57 to 2.65, which is similar to previous studies (De Vries and Georghiou 1981; Gist and Pless 1985; Pree et al. 1996).

A coarse estimate of the no-observed-adverse-effect concentration (NOEC) for population-level effects on non-target organisms can be obtained through the regression equations of Slooff et al. (1986). Consequently, the RQs using AECs for the insecticides evaluated in this study would be below the estimated NOECs. These results using AECs are supported by the field bioassays and demonstrate that a single ULV application of synergized or unsynergized permethrin most likely will not result in population or ecosystem level impacts on medium- to large-bodied insects.

The risks to medium and large-bodied insects after aerial applications are most likely lower than those estimated here because concentrations deposited on the ground are generally lower than truck-mounted ULV applications (Schleier III et al. 2008), which is supported by Boyce et al. (2007) and Kwan et al. (2009) who observed no significant mortality of these size classes after aerial applications of synergized pyrethrins. The results for permethrin are different from those of Tietze et al. (1996), who found that ULV applications of malathion caused significant mortality of sentinel crickets. However, the results of our study are supported by Davis and Peterson (2008), Kwan et al. (2009), Boyce et al. (2007), and Hill et al. (1971), showing little to no impact on medium- to large-bodied common terrestrial arthropods after single and multiple ULV applications.

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