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ACUTE EFFECTS OF PERMANONE® 31-66 (PERMETHRIN–PIPERONYL BUTOXIDE) ON NONTARGET MINNOWS AND GRASS SHRIMP

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ABSTRACT. Immature sheepshead minnows (*Cyprinodon variegatus*), inland silversides (*Menidia beryllina*), mosquitofish (*Gambusia holbrooki*), and grass shrimp (*Palaemonetes pugio*) were exposed to the mosquito adulticide, Permanone® 31-66, in a series of static toxicity tests conducted in the laboratory. At 24 h, in order of decreasing susceptibility, LC_{50} values were 0.843, 4.07, 5.46, and 6.04 ppb for grass shrimp, inland silverside, sheepshead minnow, and mosquitofish, respectively. Forty-eight-hour LC_{50} values for grass shrimp, inland silverside, sheepshead minnow, and mosquitofish were 0.049, 2.86, 3.02, and 4.29 ppb, respectively.

In recent years, pyrethroids have become increasingly popular in Florida mosquito control programs (Jones 1990). Pyrethroid adulticides, permethrin and resmethrin, have been used in mosquito control as an alternative to the standard organophosphates, malathion, naled, and fenthion. Pyrethroids are synthetic analogues of pyrethrins that have several properties favorable for use in public health spraying, such as low mammalian and avian toxicity, short to semipersistence in the environment (Hansen et al. 1983), and high target species susceptibility (Elliott et al. 1978). One problem with pyrethroids however is the hazard to nontarget fish, Crustacea, and beneficial insects following deposition in sensitive habitats.

As summarized by Clark et al. (1989), permethrin is acutely toxic to invertebrates with 96-h LC_{50}s ranging from 0.02 to 0.73 ppb for the stone crab *Menippe mercenaria* (Say), mysid *Mysidopsis bahia* Molenock, sand shrimp *Crangon septemspinosa* Say, copepod *Nitocra spinipes* Boeck, pink shrimp *Penaeus duorarum* Burkenroad, brown shrimp *Penaeus aztecus* Ives, and American lobster *Homarus americanus* Milne-Edwards. Toxicity to fish was only slightly lower, ranging from 2.2 to 12 ppb among Atlantic silverside *Menidia menidia* (Linn.), striped mullet *Mugil cephalus* (Linn.), bleak *Alburnus alburnus* Linn., and Atlantic salmon *Salmo salar* Linn. (Clark et al. 1989). Persistence in the environment is another point of concern. In soil, permethrin has a half-life of 28 days (Kaufman et al. 1977); a half-life of 14 days was measured for permethrin in seawater exposed to sunlight (Schimmel et al. 1983). Considering this level of nontarget susceptibility and the relatively photostable permethrin molecule, environmental impacts from permethrin products are a legitimate concern.

Although permethrin toxicology has received substantial attention (Anderson 1989, Clark et al. 1989, Mian and Mulla 1992), less work has been conducted using synergized formulations of permethrin. This study focuses on a single mosquito control formulation, Permanone®, containing a mixture of permethrin and piperonyl butoxide at a ratio of 31.28:66%, respectively. The goal of this study is to assess the static acute toxicity of Permanone to several common nontarget organisms that inhabit fresh and brackish water marshland habitats. The 4 species investigated in this paper occur in the shallow waters of bayous and bays of the southeastern United States and thus are potentially exposed to mosquito adulticides following aerial or ground applications.

In 1994, populations of *Cyprinodon variegatus* Lacepede, *Menidia beryllina* (Cope), *Gambusia holbrooki* Girard, and *Palaemonetes pugio* Holthuis were cultivated in field ponds at the John A. Mulrennan, Sr. Research Laboratory (JAMSRL), Panama City, FL. Eggs of the oviparous fishes were collected on substrata as described for *M. beryllina* in Tietze et al. (1992). While *M. beryllina* were hatched and maintained as fry in cages placed inside a rearing pond, *C. variegatus* eggs were hatched and fry were reared in 5-gallon buckets in the laboratory. *Gambusia holbrooki* were reared for testing as described in Tietze et al. (1991). The fry of each fish were fed newly hatched *Artemia* or 1st-instar mosquito larvae twice daily and just prior to testing. Five-day-old *C. variegatus* were also fed powdered flake food. Immature *P. pugio* were obtained from gravid females collected from both rearing ponds and Robinson Bayou adjacent to JAMSRL. *Palaemonetes pugio* were fed *Artemia* once prior to testing.

Age of test organisms ranged from 8 to 11 days for *C. variegatus,* 13–17 days for *M. beryllina,* 2–5 days for *G. holbrooki,* and <24 h for

Table 1. Concentration of permethrin (Permanone® 31-66) causing 10% and 50% mortality in the grass shrimp and 3 minnow species at 2 exposure intervals.

	Lethal concentration (ppb)			
Species	LC_{10}	CI (95%)	LC_{50}	CI (95%)
Palaemonetes pugio				
24 h	0.197	0.142–0.250	0.843	0.758–0.960
48 h	0.022	0.019–0.024	0.049	0.046–0.052
Gambusia holbrooki				
24 h	4.25	3.83–4.60	6.04	5.77–6.27
48 h	3.21	2.98–3.40	4.29	4.16–4.39
Cyprinodon variegatus				
24 h	3.69	3.48–3.88	5.46	5.30–5.63
48 h	2.08	1.96–2.18	3.02	2.93–3.11
Menidia beryllina				
24 h	3.11	2.95–3.24	4.07	3.96–4.18
48 h	2.25	1.99–2.42	2.86	2.70–3.02

P. pugio. Immature organisms have been favored for use in pesticide bioassays (ASTM 1980) because of their greater susceptibility, and homogeneity in age and because smaller-sized organisms are sometimes more easily maintained under laboratory conditions.

Permanone (31.28% AI permethrin : 66.00% AI piperonyl butoxide) was formulated in pesticide-grade acetone (American Chemical Society) to achieve the desired concentration by dispensing 1 ml of each formulation in 500 ml of water. Fish bioassays were based on 7 treatment concentrations including controls; each treatment had 6 beakers, and each beaker contained 5 organisms. In contrast, bioassays for *P. pugio* were conducted using 10 individuals/beaker and 7 concentrations, 3 of which were used for calculating 24-h-exposure LC values and 3 for the 48-h values. In each bioassay, controls were treated with 1 ml of acetone.

Bioassays were conducted using 600-ml Pyrex® beakers. Tests using *M. beryllina* and *C. variegatus* contained 500 ml of 8-ppt saltwater, whereas water used in *P. pugio* bioassays was adjusted to 15 ppt. Saltwater was pumped from Robinson Bayou into a 1,135.5-liter tank and filtered through a charcoal/diatomaceous- earth mixture. Salinity was adjusted to the desired level by the addition of deionized water. *Gambusia* bioassays were conducted using unfiltered well water. Twenty-four hours prior to each test the diluent water was placed in a 95-liter container where it was aerated and treated with ultraviolet irradiation. Beakers were suspended in a water bath using a Haake® open bath immersion heater/circulator to regulate and maintain a water temperature of 27 ± 0.5°C. A photoperiod of 16 h light and 8 h darkness was maintained with fluorescent lights. A minimum of 5 "good" bioassays (where the Probit chi-square test was not significant at $\alpha = 0.01$ and mortality of control organisms was <5%) were pooled and reanalyzed using the Probit procedure (SAS Institute, Inc. 1990) to determine the LC_{10}, LC_{50}, and the respective 95% confidence intervals for each species.

Concentrations of Permanone causing 50% mortality of young nontarget organisms during a 48-h exposure ranged from 0.049 ppb for *P. pugio* to 4.29 ppb for *G. holbrooki* (Table 1). *Palaemonetes pugio* was the most susceptible species in this study and consistently exhibited mortality at concentrations below 1 ppb. Based on LC_{10} values, minnows tolerated concentrations up to about 2 ppb (Table 1); however, above the latter concentration mortality occurred for *C. variegatus, M. beryllina,* and *G. holbrooki* (listed in order of decreasing susceptibility). In each case, a 48-h exposure yielded a significantly ($P < 0.05$) lower LC value than that of a 24-h exposure, as based on nonoverlapping 95% confidence intervals (Table 1).

Compared to the other species tested (Fig. 1), Permanone was more acutely toxic to *P. pugio* as evidenced by the steeply sloped probit lines (Fig. 2). In addition, this species displayed the greatest increase in susceptibility with exposure interval (Table 1 and Fig. 2). A 17-fold increase in toxicity occurred when the 48-h LC_{50} value was compared to that of a 24-h exposure. In comparison, residual toxicity for minnows ranged from 1.4 to 1.8× at similar exposure times.

Hansen et al. (1983) studied the toxicity of

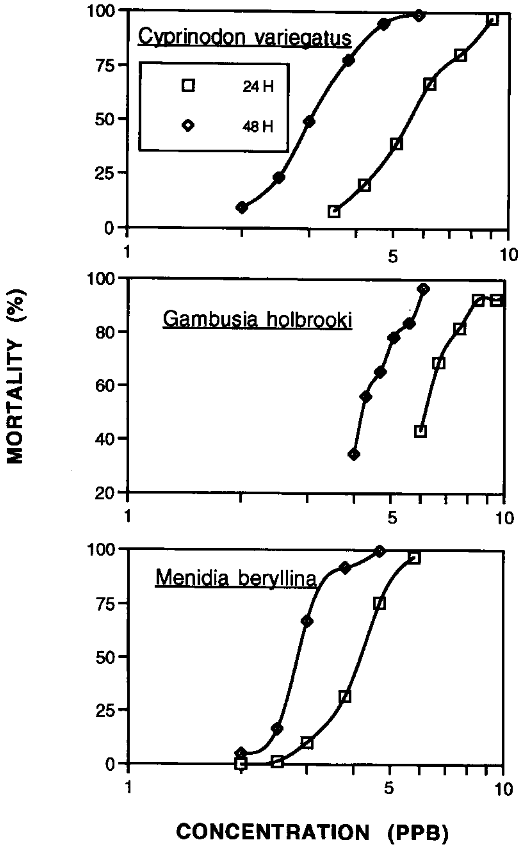

Fig. 1. Dose–response curves for sheepshead minnows (*Cyprinodon variegatus*), mosquitofish (*Gambusia holbrooki*), and inland silversides (*Menidia beryllina*) exposed to Permanone 31-66 for 24- and 48-h intervals.

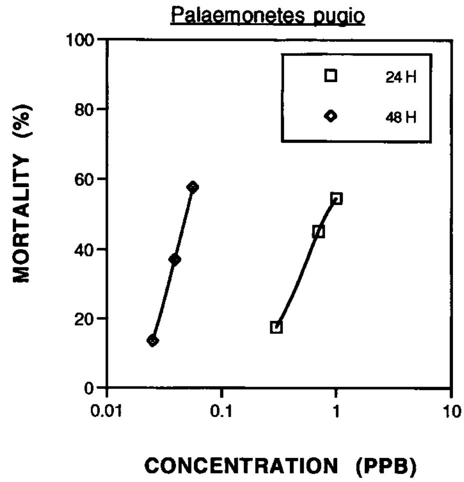

Fig. 2. Dose–response curves for grass shrimp (*Palaemonetes pugio*) exposed to Permanone 31-66 for 24- and 48-h intervals.

permethrin to young sheepshead minnows using an intermittent flow-through system. They found that permethrin concentrations above 22 ppb reduced survival of newly hatched fish whereas concentrations less than 10 ppb had no deleterious effects. These values are higher than those of the current study, which may be due to differing experimental regimes; static vs. flow-through systems. In another study, immature topsmelt (*Atherinops affinis* [Ayres]) and inland silversides (*M. beryllina*) were exposed to permethrin in 96-h static toxicity tests resulting in LC_{50}s of 25.3 and 27.5 ppb, respectively (Hemmer et al. 1992). Greater tolerance in the latter study may be attributed to the use of older test fish, 38 and 30 days old for *A. affinis* and *M. beryllina,* respectively. The toxicity of permethrin to newly hatched crayfish (*Procambarus clarkii* [Girard]) based on the 96-h LC_{50} value was 0.39 ppb (Jolly et al. 1978), substantially higher than the 48-h LC_{50} value of *P. pugio* in the current study. In the latter study, mature mosquitofish had a 96-h LC_{50} value of 15 ppb, again appreciably greater than that of the current study.

Pesticide drift and deposition into wetland habitats continue to be important concerns when using mosquito adulticides. Ironically, whereas drift is required to expose the target species and achieve successful mosquito reduction, unintentional drift and deposition into sensitive wetlands, frequently the original source of mosquito production, may be logistically unavoidable. To help prevent the latter scenario, pesticide "use precautions" on the Permanone label state the product should not be used within 30 m of lakes and streams. Droplets generated by cold aerosol machines are between 1 and 17 μm in volume median diameter, a range in size expected to drift much farther than 30 m (Tietze et al. 1994). Buffer zone width for ground-based applications of permethrin was assessed by Payne et al. (1988) for drift in sensitive aquatic habitats by measuring deposition at various distances from the point of application and using *Aedes aegypti* (Linn.) as a bioindicator. Buffer width was estimated by choosing an acceptably low mortality and determining the downwind distance at which this value was obtained. Using this technique, a 20-m swath width limited mortality in *A. aegypti* and rainbow trout (*Salmo gairdneri* Richardson) populations to 10 and 0.1%, respectively. Theoretical deposition for Permanone, based on maximum allowable ground application rates within a 0.405-ha "target zone" (using 7.84 g AI/ha and assuming a homogeneous distribution of a single 91.4-m swath), was calculated to be 78.4 ng/cm^2. Assuming permethrin droplet dynamics are similar to those of malathion (Tietze et al. 1994), maximum deposition within the target zone may be estimated to be 10% of the expected rate or 7.8 ng/cm^2. The latter deposition rate on 15.2 cm of water would cause a hypothetical concentration of 0.51 ppb. Mortality exceeding 10% of young *P. pugio,* based on these static laboratory bioassays, would be expected following exposure to this concentration. Mortality of young minnows is not expected at that concentration. Further investigations measuring deposition of permethrin resulting from field applications will be needed to substantiate these initial laboratory predictions. Additional research should also focus on nontarget effects of other synergized formulations of permethrin and piperonyl butoxide using the various commercially available ratios of the above pesticide and synergist.

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