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Acute Toxicity of Permethrin/Piperonyl Butoxide on Hybrid Striped Bass

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Mosquito control agencies in the Mid-Atlantic region are currently choosing pyrethroids, such as permethrin, over traditional organophosphates, such as malathion, more frequently in public agency mosquito control operations. Permethrin has many characteristics that favor its use as a mosquito/biting fly adulticide including: high target species toxicity (Elliott et al. 1978), low mammalian and avian toxicity, and relatively short environmental half-life (Hansen et al. 1983). Permethrin has a half-life of 28 days (Kaufman et al. 1977) in soil and 14 days in seawater exposed to sunlight (Schimmel et al. 1983). It degrades rapidly in water but accumulates (bioconcentrates) in fish (Wei et al. 1995). Kreutzweiser & Wood (1991) measured a median bioconcentration factor of 98 (mean=148) in stream fishes.

The major contraindication to permethrin use is relatively high toxicity to non-target organisms including macroinvertebrates (ranging from 0.02 to 0.73 ppb in species tested) and estuarine fishes (ranging from 2.2 to 12 ppb in species tested) (Clark et al. 1989). Exposure to permethrin, a synthetic pyrethroid, elicits a neurological response characterized by restlessness, loss of coordination, systemic tremors, and paralysis in most species tested, including insects and fish. Eells et al. (1993) compared permethrin-induced neuronal responses in fish and rats and the results suggest species-specific responses since maximal membrane depolarizations were three times greater in fishes than in rats using comparable doses. This provides evidence that neurotoxin binding properties differ in fish and mammals and supports previously reported findings of low mammalian toxicity of permethrin.

For insect control, permethrin is commonly synergized with piperonyl butoxide (PBO). While this formulation is more commonly applied than unsynergized permethrin in mosquito control agents, the synergized formulation has not yet been well studied. However, Tietze et al. (1995) used Permanone (permethrin and PBO at a ratio of 31.28 : 66 %) to test several common nontarget organisms that inhabit fresh and brackish water marshlands. PBO is a metabolic inhibitor (Feng et al. 1995), and may exhibit insect juvenoid growth regulator activity (Satoh et al. 1995). PBO decreased permethrin resistance in houseflies (depending on genetic line) and in the German cockroach (Cochran 1994), thus increasing the apparent

toxicity of permethrin. PBO increases the efficacy of permethrin by blocking the enzymatic detoxification processes of permethrin by the insect. Unsynergized permethrin produces rapid knockdown properties, typically followed by a substantial recovery period for a large portion of the treated population. PBO reduces this recovery and is commonly used to treat populations of insects believed to have developed chemical resistance. Furthermore, the addition of PBO as a synergist allows for decreased rates of application of the primary active ingredient while maintaining equal or improved mortality. An effective synergistic ratio of PBO:permethrin is 1:1 active ingredient (AI) (*Meisch MV pers. comm., Dept. Entomology, Univ. AR, Fayetteville*).

The purpose of this investigation was to determine the acute toxic effects of PBO synergized permethrin (1:1, by AI) on juvenile striped bass hybrids (*Morone saxatilis* x *Morone chysops*), an important aquaculture species, using standard static acute toxicity testing procedures (Amdur et al. 1991).

MATERIALS AND METHODS

Concentrations of 0.0, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 ppb of the synergized mixture were tested to determine the lethal concentration (LC) that caused 50% and 90% hybrid striped bass mortality after 24, 48, 72, and 96 hr. Three replicates were used for each concentration. Fish of the same cohort were obtained from AquaFuture (Turner's Falls, MA). Juvenile fish were used because of their greater susceptibility to toxicants, ease of testing in the laboratory, and homogeneity of age. Fish weights and total lengths were recorded at the end of the 96 hr monitoring period. Fish were fed %x/day, approximately 3% of total body wet weight, with Perdue Farms Striped Bass Food (Perdue Farms, Catawissa, PA).

Two tests were conducted. In the first test (24 hr acute toxicity test), fish were exposed to their assigned concentration of synergized permethrin for 24 hr, removed to clean filtered water, and observed for an additional 72 hr. Mortality and behavioral observations were made at 96 hr. In the second test (96 hr acute toxicity test), fish remained in their assigned concentrations for 96 hr and mortality and behavioral observations were made at 24, 48, 72 and 96 hr. Although controls were reused, no fish exposed to pesticide in the first test was reused in the second test.

Ten gallon (37.85 L) aquaria were filled with 32.50 L well water and aerated for 24 hr to remove excess nitrogen. Salinity was adjusted to 10-11 ppt with Forty Fathoms Crystal Sea artificial sea salt (Marine Enterprises International, Baltimore, MD). Temperature was maintained at 18.8-19.0" C. Water hardness (Ca^{++}) varied between 220-270 ppm in the 24 hr exposure tanks and between 250-300 ppm in the 96 hr exposure tanks. The 1ight:dark ratio of 9.5:14.5 was ambient for the testing period.

A 16 ppm stock solution was made by adding 50 µL PBO (McLaughlin, Gormley, King, Co., Minneapolis, MN) and 88 µL Punt 57-OS (57 % Permethrin AI, Bonide Products, Yorkville, NY) to 4.9 ml 99.5 % research grade acetone (Aldrich Chemical Co., Milwaukee, WI) + 6.245 L well water. This produced a solution of PBO and permethrin at a 1:1 AI ratio by weight. The solution was agitated for several minutes to ensure homogeneity. Nominal test solutions of 0.25-32.0 ppb were produced by adding the appropriate amount of 16 ppm stock solution to 32.5 L salt water (after removing an equivalent amount of salt water). Solvent controls received the same volume of acetone introduced to the highest test concentration (32.0 ppb): 9.8 µL in 32.50 L.

Fish were acclimated to the testing facility for 3 days before the 24 hr test and 11 days before the 96 hr test. Prior to toxicity testing, fish were randomly placed into one of the 9 test concentration tanks and allowed to acclimate for 24 hr. The 24 hr acute toxicity test began 7 December 1997 at 1300 hr and terminated 11 December 1997 at 1300 hr. The cohort was approximately 80 days old at the inception of the study. A total of 162 fish were tested; 6 fish in each of three replicates, 18 fish per test concentration. Mortality and behavioral observations were made at 96 hr.

The 96 hr acute toxicity test began 15 December 1997 at 1030 hr and terminated 19 December 1997 at 1030 hr. The cohort was approximately 88 days old at the inception of the study. A total of 135 fish were tested; 5 fish in each of three replicates, 15 fish per test concentration. Mortality and behavioral observations were made at 24, 48, 72 and 96 hr. Probit analysis (SYSTAT, ver 7.0, SPSS Inc., Chicago, IL) provided a statistically-based estimate of LC_{50} and LC_{90} values. The LC_{90} was determined by utilizing non-linear estimation techniques.

Agitated/excited fish exhibited spontaneous twitching, generalized clonic contractions, and swam excessively, typically at exaggerated speeds. Ataxic fish were characterized by failure to maintain equilibrium, uncoordinated swimming, systemic tremors, systemic tetany, and/or paralysis.

RESULTS AND DISCUSSION

Fish in both tests manifested agitated/excited behavior and ataxia, characteristic of sublethal toxicity. All mortality and sublethal behavioral effects occurred in the 16 and 32 ppb concentrations and none at the 0.0 (solvent control), 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 ppb concentrations of the synergized permethrin. There was minimal variability in the distribution of mortality and sublethal behaviors between replicates.

Total mean length of fish used in the 24 hr exposure was 57.7 mm (SD = ± 6.7, n=162), the mean weight was 2.5 ± 0.8 g. Observations were made at 96 hr after

initial exposure, and mortality is recorded in Table 1. Of the 18 fish tested at each concentration, two died at 16 ppb and 12 at 32 ppb. Five fish exposed to a concentration of 16.0 ppb and six fish exposed to a concentration of 32.0 ppb (ah those still alive) exhibited sublethal toxicity. The LC_{50} was 26.7 ppb, and the LC_{90} was 45.2 ppb (Table 2).

Table 1. Acute PBO/permethrin toxicity.

nominal conc (ppb)	24 hr exposure (n = 18) # dead 24 hr	96 hr exposure (n = 15) # dead 24 hr	48 hr	72 hr	96 hr
0	0	0	0	0	0
0.25	0	0	0	0	0
0.50	0	0	0	0	0
1.0	0	0	0	0	0
2.0	0	0	0	0	0
4.0	0	0	0	0	0
8.0	0	0	0	0	0
16.0	2	0	6	7	7
32.0	12	6	15	15	15

Total mean length of fish used in the 96 hr exposure was 57.9 mm (SD = ± 6.5, n = 135), the mean weight was 2.4 ± 0.8 g. Observations were made at 24, 48, 72 hr and 96 hr and mortality is recorded in Table 1. After 24 hr exposure, seven fish exposed to a concentration of 16.0 ppb and nine fish (all those still alive) exposed to a concentration of 32.0 ppb exhibited sublethal toxicity. After 48 hr, the remaining 9 fish in the 16.0 ppb concentration exhibited sublethal toxicity. All of the fish in the 32 ppb concentration were dead by 48 hr. At 72 hr, one additional fish had died in the 16.0 ppb concentration. There was no further mortality after 72 hr. None of the fish at any of the other concentrations exhibited deleterious effects (Table 2).

Agitated/excited behavior was not observed in the 24 hr test but appeared in the 96 hr exposure test. Eleven fish exhibited agitated behavior at some point in the 96 hr study. Three fish that showed agitated behavior eventually died, 1 fish became agitated after 48 hr and was still agitated at 96 hr, and seven appeared normal at the conclusion of the test.

Ataxic behavior was observed in five fish at 16.0 ppb and six fish at 32.0 ppb in the 24 hr study and in five fish at 16.0 ppb and nine fish at 32.0 ppb in the 96 hr study. Twelve fish subsequently died, one that was initially agitated became ataxic but eventually returned to an agitated condition, another was initially agitated, became ataxic, and subsequently appeared normal.

Table 2. Estimates of LC_{50} and LC_{90} values for 24 and 96 hr exposures

Estimates of LC_{50} and LC_{90} for 24 hr exposure / observation at 96 hr in ppb

Parameter	Asymptotic Estimate	S.E.	Confidence Interval Lower < 95%> Upper
L C_{50}	26.7	2.7	21.4 32.1
L C_{90}	45.2	8.9	27.8 62.6

Estimates of LC_{50} and LC_{90} for 24 hr mortality in 96 hr exposure in ppb

Parameter	Asymptotic Estimate	S.E.	Confidence Interval Lower < 95%> Upper
L C_{50}	32.9	1.2	30.6 35.2
L C_{90}	37.8	1.4	35.0 40.6

Estimates of LC_{50} and LC_{90} for 48 hr mortality in 96 hr exposure in ppb

Parameter	Asymptotic Estimate	S.E.	Confidence Interval Lower < 95%> Upper
L C_{50}	16.4	0.5	15.4 17.5
L C_{90}	18.7	0.6	17.5 19.9

Estimates of LC_{50} and LC_{90} for 72/96 hr mortality in 96 hr exposure in ppb

Parameter	Asymptotic Estimate	S.E.	Confidence Interval Lower < 95%> Upper
L C_{50}	16.1	0.5	15.1 17.2
L C_{90}	18.4	0.6	17.2 19.6

Fish in both tests exhibited sublethal toxicity. Agitated behavior was the less severe manifestation; 27 % of the affected fish died while 86 % of the fish exhibiting ataxic behavior eventually succumbed.

Tietze et al. (1995) used Permanone (synergized permethrin and PBO at a ratio of approximately 1:2) to test several common nontarget organisms that inhabit fresh and brackish water marshland habitats. They tested grass shrimp (*Palaemonetes pugio*), Southern silversides (*Menidia beryllina*), sheepshead minnows (*Cyprinodon variegatus*), and mosquitofish (*Gambusia holbrooki*) and obtained 24 hr LC_{50} values of 0.843, 4.07, 5.46, and 6.04 ppb respectively and 48 h LC_{50} values of 0.049, 2.86, 3.02, 4.29 ppb respectively. This test, using a 1:1 AI ratio, found a 24 hr value for juvenile hybrid striped bass of 27 ppb (24 hr exposure, observation at 96 hr) and a 24 h LC_{50} value of 32.8 ppb (24 hr exposure, observation at 24 hr).

There have only been a few other tests using closely related compounds on fish. Hansen et al. (1983) tested permethrin on sheepshead minnows and found that concentrations greater than 22 ppb reduced survival of newly hatched fish but concentrations less than 10 ppb had no lasting effects. However, that study used an intermittent flow-through system and tested unsynergized permethrin. Hemmer et al. (1992) used permethrin in a 96 hr static toxicity test of immature topsmelt (*Atherinops affinis*) and silversides and obtained LC_{50} values of 25.3 and 27.5 ppb. These fish were older than those used in Hansen's study (38 and 30 days old respectively). Jolly et al. (1978) obtained a 96 hr LC_{50} value of 15 ppb of permethrin on mature mosquitofish. Our study used synergized permethrin on juvenile striped bass hybrids of similar size and age and mortality was of the same order of magnitude. Lower concentrations caused sublethal effects but some of the fish did eventually recover.

Kreutzweiser & Wood (1991) found that aerial applications of permethrin at 17.5 g ha^{-1} resulted in maximum concentrations of 0.07-2.5 ppb in stream water. The median maximum concentration was 0.54 ppb. The mean half-life was 10.3 hr at oversprayed sites. Approximately 50% of fish from streams in treated areas contained measurable residues (> 5 ppb) up to 28 days post treatment. No residues were detected in fish at 69 or 73 days after the applications. In fish that contained measurable residues, the median bioconcentration factor was 98 (mean=148). Because fish exhibit bioconcentration, the use of synergized permethrin as a mosquito adulticide and drift from aerial spraying may endanger sensitive aquatic species.

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