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# Toxicity of Three Mosquito Insecticides to Crayfish

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Since 1990 the pyrethroid insecticide resmethrin has been used in New York State (NYS) to control adult black flies and mosquitoes. More recently two additional synthetic pyrethroids, permethrin and sumithrin were added to the list of labeled adulticides (USEPA 2002a). All three pyrethroid insecticides have the synergist piperonyl butoxide (PBO) added to their formulations. Toxicity studies have shown these three adulticides to be very toxic to fish and other aquatic life (Paul et al. 2005).

Mosquito control has become more widespread in NYS and across the US due to the spread of West Nile Virus, a disease transmitted by mosquitoes (CDC 2003). Very large areas are being treated with pyrethroid insecticides and other mosquito control agents. This increased use has exposed more areas to potential non-target effects from these pesticides.

This study determines the toxicity of these pesticides to crayfish, a widely distributed, important invertebrate in many aquatic systems. In addition, we determined the effect of the synergist PBO on the toxicity. We further evaluate the possible effect of multiple exposures, since mosquito control programs can conduct multiple applications with short periods of time between applications.

## MATERIALS AND METHODS

The resmethrin and permethrin products used in our study are produced by Aventis Environmental Science USA LP (Montvale, NJ). The resmethrin formulations are Scourge 4% + 12% MF™ containing 4.14% w/w resmethrin and 12.42% w/w PBO. Aventis also prepared a special non-synergized Scourge which contained all of the components of Scourge 4% + 12% except the PBO. The permethrin formulations used are Permanone 31-66™ which contains 31.28% w/w permethrin and 66% w/w PBO and Permanone Technical Insecticide™ with 92% w/w permethrin.

Anvil 10+10™ is a synergized formulation containing 10% w/w sumithrin and 10% w/w PBO, and formulated by Clarke Mosquito Control Products (Roselle, IL). The non-synergized formulation was Sumithrin Technical Insecticide containing 96.8% w/w sumithrin and produced by McLaughlin Gormley King Company (MGK®) (Minneapolis, MN).

Spring water from the NYS Department of Environmental Conservation (DEC) Rome Fish Hatchery was used as the dilution water in test solutions (pH = 8.10, hardness = 132 mg/L

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$CaCO_3$, alkalinity = 117 mg/L $CaCO_3$, conductivity = 299 µmho/L). Temperature was held constant (16.5±1.0°C) using a water bath in all tests except where noted below. Dissolved oxygen was monitored daily and was never less than 6.0 mg/L.

Fresh stock solutions of each formulation were prepared for each test conducted. Stock solutions were mixed by dissolving the insecticides in acetone to make 120,000 µg/L resmethrin, permethrin, or sumithrin. These stock solutions were used to apply the pesticides to the study chambers. Control containers were mixed with a volume of acetone equal to that of the highest test concentration. All testing was conducted in 20-L glass containers with 16 L of test solution.

The crayfish selected for most tests was *Orconectes immunis*, a species native to NYS. They were obtained from walleye culture ponds at the DEC South Otselic Fish Hatchery while the culture ponds were being drained. Crayfish were held in tanks for a minimum of two weeks prior to use in testing at the DEC Aquatic Toxicant Research Unit (Rome, NY). Only crayfish of apparent good health, in good condition, and which exhibited normal responses to stimuli were used in testing. Crayfish tested had a mean weight of 2 g (range 1-3 g) except as noted below.

Standard 96-hr static toxicity testing was selected to evaluate and compare the toxicity of the three pyrethroid insecticides to crayfish (USEPA 2002b). While static tests have limitations, they are the most commonly performed tests, allowing for the greatest number of comparisons to other tests. In addition, the accidental spraying of ponds or marshes with these pesticides is unlikely to result in the continuous addition of insecticide to the field. Counts of dead and affected crayfish were made at 3, 24, 48, 72, and 96 hr. Affected crayfish were those which showed signs of intoxication including erratic swimming/crawling and an inability to remain upright. Dead crayfish were removed and weighed. Crayfish were not fed during exposure to insecticides, and all crayfish were weighed at the end of the tests. We calculated LC50 and EC50 values using nominal concentrations and the trimmed Spearman-Karber method (Hamilton et al. 1977).

Testing conducted with resmethrin formulations used concentrations of 0, 0.080, 0.220, 0.604, 1.656, and 4.545 µg/L resmethrin. Five crayfish were exposed in each test container. There were 9 replica of each concentration so that 45 crayfish were exposed to each concentration for each formulation.

Tests conducted with sumithrin formulations used concentrations of 0, 0.029, 0.080, 0.220, 0.604, 1.656, 4.545, and 12.47 µg/L sumithrin. Again, five crayfish were exposed in each test container. Six replica of each concentration resulted in a total of 30 crayfish exposed per concentration for each sumithrin formulation.

Testing conducted with permethrin formulations used concentrations of 0, 0.011, 0.029, 0.080, 0.220, 0.604, and 1.656 µg/L permethrin. Five crayfish were exposed in each test container. In this case there were 8 replica of each concentration, therefore, 40 crayfish were exposed to each concentration for each permethrin formulation.

We conducted a series of static crayfish tests with two species of larger crayfish, *O. immunis* obtained from the hatchery, and *Procambarus clarkii* obtained from a commercial supplier in Louisiana. Both of these species of crayfish had a mean weight of

14 g (range 9-21 g). These larger crayfish were tested with the synergized sumithrin only. The concentration series used was 0, 0.220, 0.604, 1.656, 4.545, and 12.47 µg/L sumithrin. Three crayfish were placed into each exposure chamber. There were 10 replica of each concentration so that 30 crayfish were exposed to each concentration. Testing of larger *O. immunis* was conducted at 17.0 ± 0.7°C. Testing of *P. clarkii* was conducted at 10.0 ± 0.5°C.

The last experiment which we conducted was a repeat exposure experiment. This was conducted using synergized resmethrin and the smaller sized *O. immunis*. Test crayfish were divided into 3 groups and exposed for 24 hr to 0 (solvent control), 0.220, or 0.604 µg/L (3 replica of each). Crayfish were then held in tanks with flowing dilution water for 1 week and were monitored daily. Each group of crayfish were equally redistributed into a second 24-hr exposure at 0, 0.220, and 0.604 µg/L. Following the second exposure, the crayfish were held and monitored for an additional week. This led to 9 possible exposure regimens (see Table 1). All of these experiments were conducted at 13.0 ± 0.3°C These experiments were repeated three times, for a total of 30 crayfish exposed to each regimen. Dead crayfish were removed and weighed. All remaining live crayfish were weighed at the end of the experiment.

## RESULTS AND DISCUSSION

We found all three of the pyrethroid active ingredients to be highly toxic to crayfish (Table 2). All three insecticides are similar in their toxicity to crayfish especially during the first 48 hr of exposure. Permethrin and sumithrin appear to continue to exert some impact after 48 hr, while resmethrin appears to lose potency. The lowest observed adverse effect concentration (LOAEC) for the synergized formulations of permethrin, resmethrin, and sumithrin were 0.029, 0.220, and 0.080 µg/L respectively. Smaller crayfish were clearly more sensitive (by an order of magnitude) to sumithrin than larger crayfish of the same species. We also found that *P. clarkii* were more sensitive to sumithrin than same size *O. immunis* by a factor of 2-4, but the *P. clarkii* were tested at cooler temperatures. Pyrethroid insecticides are known to be more toxic to terrestrial insects and fish at lower temperatures (Bradbury and Coats 1989), therefore the difference we observed may be due to a difference in test temperatures.

The presence of the synergist PBO significantly increased the lethality of permethrin, but showed no clear pattern for resmethrin and sumithrin. While the difference in toxicity

**Table 1.** Percent survival of NYS crayfish *Orconectes immunis* following repeat 24-hr exposures to synergized resmethrin. One week between each exposure.

| | First Exposure Concentration (µg/L) | | |
|---|---|---|---|
| Second Exposure Concentration (µg/L) | 0.000 | 0.220 | 0.604 |
| 0.000 | 100 | 86 | 77 |
| 0.220 | 86 | 70 | 47 |
| 0.604 | 77 | 20 | 0 |

**Table 2.** LC50s and EC50s (95% confidence limits) from standard toxicity tests of synergized and non-synergized sumithrin, permethrin, and resmethrin to small and large crayfish. *O. imm* = *Orconectes immunis*. *P. clark* = *Procambarus clarkii*. NA indicates that EC50 was not available.

| Formulation | Species/ Size | Lethal/Effective Concentration (µg/L) (95% Confidence Interval) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 3 hr | 24 hr | | 48 hr | | 96 hr | |
| | | EC50 | LC50 | EC50 | LC50 | EC50 | LC50 | EC50 |
| Synergized Permethrin | *O. imm* small | 0.35 (0.31-0.38) | 0.46 (0.38-0.55) | 0.18 (0.15-.21) | 0.24 (0.20-0.28) | 0.12 (0.10-0.13) | 0.10 (0.09-0.12) | 0.05 (0.04-0.06) |
| Non-synergized Permethrin | *O. imm* small | 0.46 (0.39-0.53) | 0.53 (0.43-0.67) | 0.17 (0.14-0.20) | 0.31 (0.26-0.36) | 0.11 (0.09-0.13) | 0.21 (0.17-0.25) | 0.08 (0.07-0.11) |
| Synergized Resmethrin | *O. imm* small | 0.36 (0.34-0.39) | 0.79 (0.61-1.01) | 0.21 (0.17-0.25) | 0.49 (0.40-0.60) | 0.23 (0.19-0.27) | 0.42 (0.35-0.50) | 0.31 (0.27-0.35) |
| Non-synergized Resmethrin | *O. imm* small | 0.36 (0.34-0.39) | 0.91 (0.70-1.16) | 0.25 (0.21-0.30) | 0.55 (0.45-0.66) | 0.25 (0.21-0.30) | 0.51 (0.42-0.62) | 0.32 (0.28-0.36) |
| Synergized Sumithrin | *O. imm* small | 0.38 (0.34-0.42) | 0.61 (0.50-0.76) | 0.20 (0.17-0.24) | 0.35 (0.28-0.43) | 0.22 (0.19-0.27) | 0.24 (0.20-0.29) | 0.20 (0.16-0.26) |
| Non-synergized Sumithrin | *O. imm* small | 0.35 (0.31-0.39) | 0.61 (0.50-0.76) | 0.14 (0.10-0.19) | 0.40 (0.33-0.48) | 0.15 (0.11-0.21) | 0.35 (0.29-0.43) | 0.19 (0.13-0.27) |
| Synergized Sumithrin | *O. imm* large | 3.03 (2.21-4.18) | 7.53 (5.57-10.2) | 1.23 (0.74-2.04) | 3.37 (2.02-5.61) | 1.23 (0.74-2.04) | 2.62 (1.50-4.57) | 1.78 (1.19-2.67) |
| Non-synergized Sumithrin | *P. clark* large | NA | 4.06 (2.59-6.38) | 0.82 (0.68-0.99) | 1.18 (0.95-1.48) | 0.74 (0.61-0.89) | 0.71 (0.59-0.86) | 0.69 (0.58-0.83) |

between the synergized formulation and technical was statistically significant, the greatest difference in toxicity was only a factor of two. This was in contrast to our studies with trout, where PBO increased the toxicity of these three insecticides, especially in short, 6-hr exposures (Paul et al. 2005).

Repeat exposures to synergized resmethrin demonstrated that prior exposure to the pesticide made the crayfish more vulnerable to the toxic effects of the second exposure (Table 1). Regardless of whether a single exposure to the 0.220 and 0.604 µg/L occurred in the first or second week, exposure for 24 hr yielded 86 and 77 % survival, respectively. Crayfish exposed to the lower concentration first, followed after one week by the higher concentration had lower survival than those which were exposed to these concentrations in reverse order ($p=0.021$). Repeat exposure after 1 week to synergized resmethrin at the higher concentration of 0.604 µg/L for 24 hr resulted in no crayfish surviving to the end of the monitoring period (1 week beyond second exposure).

Our study demonstrates that crayfish are among the most sensitive freshwater species to these pyrethroid insecticides. LC50s for permethrin are reported to be between 1 and 15 µg/L for a number of different freshwater fish (Jolly et al. 1978, McLeese et al. 1980, Paul et al. 2005). Crayfish are 1-2 orders of magnitude more sensitive. *Daphnia* 48-hr LC50s are 0.32 to 9.9 µg/L according to the EPA Pesticide Ecotoxicology Database (also known as EEDB) (Office Pesticide Programs 2000). Other research reports *Daphnia* LC50s of 0.30 to 28 µg/L (Sibney and Kaushik 1991). Our crayfish results indicate that crayfish are equally to slightly more sensitive than *Daphnia*. McLeese et al.(1980) determined a 96-hr LC50 to adult lobster (*Homarus americanus*) of 0.73 µg/L. This value is somewhat higher than the LC50 we determined for *O. immunis*, but their lobster were adult animals (450 g), whereas our test crayfish were juveniles.

The LC50s which we found for permethrin compare well with crayfish tests conducted by other researchers. Thurston et al. (1985) report a 96-hr LC50 < 1.2 µg/L to *O. immunis*. Jolly et al. (1978) found 96-hr LC50s of 0.39 µg/L for newly hatched and 0.62 µg/L for juvenile *P. clarkii*. Our permethrin data are also supported by Coulon (1982) who reports a 24-hr LC50 of 0.49 µg/L to *P. clarkii*, which is in good agreement with our 24-hr data for young crayfish. Jarboe and Romaire (1991) report 96-hr LC50s ranging from 0.438 to 1.298 µg/L for 4 different size classes of *P. clarkii*. Poirier and Surgeoner (1988) exposed crayfish (*Orconectes sp.*) for one hour to various concentrations of permethrin and monitored mortality for 47 hr following this exposure. They determined an LC50 of 3.0 µg/L from this 1-hr exposure - 48 hr post exposure mortality check. This value compares favorably with our 3-hr exposure EC50 of 3.03 µg/L. The EEDB contains a 96-hr LC50 record for the crayfish *Procambarus blandingii* of 210 µg/L. This particular value seems unusually high compared to LC50s reported by others as well as the results of our own studies.

Sumithrin may be somewhat less toxic to fish than the other two pyrethroids tested. LC50s for sumithrin are reported to be a between 1.4 and 18 µg/L for several different fish (Office Pesticide Programs 2000). Paul et al. (2005) found the 96-hr LC50s of 18.5 and 10.5 µg/L for a technical formulation of sumithrin to brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) respectively. Paul (2004) reports a 48-hr EC50 = 7.1 µg/L for a synergized formulation of sumithrin (Anvil 10+10) to *Daphnia magna*. The only other acute crustacean toxicity study for sumithrin is for the mysid *Mysidopsis bahia*.

This 96-hr EC50 = 0.025 µg/L indicating that mysids are very sensitive to this insecticide (Office Pesticide Programs 2000). The decreased sensitivity (larger LC50s) of the larger crayfish we observed is consistent with many studies of fish as well as crayfish exposed to permethrin (Jarboe and Romaire 1991).

The EEDB reports 96-hr LC50s for resmethrin in the range of 0.50 to 16.6 µg/L to a variety of fish species. These values are supported by our previous research (Paul and Simonin 1995). The EEDB reports *Daphnia* 48-hr LC50s which range from 3.1 to 110 µg/L. Additional *Daphnia* testing appears to be unavailable. The 96-hr LC50 of resmethrin to the shrimp *Penaeus duorarum* is 1.2 µg/L (Office Pesticide Programs 2000), which compares favorably with our crayfish results. Holck and Meek (1987) found a 96-hr LC50 of 0.82 µg/L for synergized resmethrin (Scourge) to juvenile *P. clarkii*. Holck and Meek (1987) also tested the synergized resmethrin against fourth instar larvae of three different species of mosquitoes. They found that crayfish had an LC50 which was an order of magnitude less than the LC50s for three species of mosquitoes.

Our repeat exposure to synergized resmethrin experiments show that crayfish which had previously been exposed were more susceptible than unexposed crayfish. Even crayfish which appeared to have fully recovered from the exposure (i.e. in good health and responding normally) were more sensitive to the second exposure. Since mosquito spray programs can be conducted with as little as 1 day between applications, crayfish may be at greater risk than what would be predicted from typical toxicity tests. We selected 1 week between exposures as a reasonable time between two applications, but even after 1 week exposed crayfish remained much more vulnerable.

Field application of these three mosquito adulticides to water at maximum application rates may result in total or near total mortality of crayfish. Crayfish primarily inhabit shallow waters (Pennak 1989). In the Southeastern US crayfish are frequently cultured in shallow flooded (0.1 to 0.5 m) rice fields (Eversole and McClain 2000). Maximum application rates for permethrin and resmethrin (7.84 g/ha) and sumithrin (3.92 g/ha) in a pond 0.3 m (1 ft) deep would yield concentrations of 2.6 µg/L and 1.3 µg/L, respectively, which are well above lethal limits. Label restrictions do not permit their application over water, but drift or accidental application over water may occur. Crayfish in streams are also frequently located in shallow depths (Pennak 1989). Colquhoun et al. (1984) considered the accidental spraying of a small stream (mean depth 0.2 m) with an organophosphate adulticide. Using their scenario, toxic concentrations of permethrin, resmethrin, and sumithrin would be present in the stream. However, in many small watersheds the overhead canopy reduces the amount of material that may enter the water. Yet, the 3-hr EC50 values for crayfish would be reached if as little as 10% of permethrin and resmethrin, or 20% of sumithrin were to enter this stream; and some mortality may be expected.

Poirier and Surgeoner (1988) reported that exposure to concentrations of permethrin of 0.5 µg/L resulted in increased invertebrate drift (including crayfish) in streams. Jarboe and Romaire (1995) demonstrated that crayfish in earthen ponds exposed to nominal concentrations of permethrin of 1-3 µg/L decreased in abundance by 54 to 83% after 7 days. These studies, when coupled with the results of our toxicity tests and those of other researchers, indicate that pyrethroid insecticides have the potential to harm crayfish in the field, even if small amounts enter the aquatic environment and are only present for a short

period of time. It remains critical to keep these pesticides out of the water when they are used.

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