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Comparison of the Toxicity of a Synergized and Non-Synergized Insecticide to Young Trout

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The use of pyrethroid insecticides is increasing (Elliott 1989; Mueller-Beilschmidt 1990). These insecticides tend to have short half-lives in the environment and as a class, have very low toxicities to birds and mammals (WHO 1989). Unfortunately pyrethroids are very toxic to fish and other aquatic organisms (Demoute 1989). Resmethrin is a type one pyrethroid which is used as a mosquito and blackfly adulticide. It is available in several formulations, some of which contain the synergist piperonyl butoxide (PBO). PBO increases the lethality of a particular dose of resmethrin in target species (Casida 1980). The magnitude of the effect of the synergist on the toxicity of resmethrin to aquatic organisms is largely unknown. The action of this synergist on non-target aquatic organisms is likely to be similar to its action on target insects (Reinbold and Metcalf 1976).

This study compares the toxicity of two formulations of resmethrin using 96-hr toxicity tests, short exposure tests, and toxicity tests of aged solutions of these insecticides. One formulation contains the synergist PBO, and the other is a non-synergized formulation.

MATERIALS AND METHODS

The two resmethrin formulations used in this study are produced by Roussel Bio Corporation (Englewood Cliffs, New Jersey). SBP-1382 insecticide 4.22 MF is a non-synergized formulation which contains 4.22% w/w resmethrin. Scourge 4% + 12% MF is a PBO synergized formulation of resmethrin which contains 4.14% w/w resmethrin and 12.42% w/w PBO. Each insecticide is a "ready-to-use" formulation and does not require further dilution prior to application. We also conducted some testing on a Scourge blank that contained PBO but no resmethrin.

Trout used in these tests were Rome Lab strain domestic brook trout (Salvelinus fontinalis) and brown trout (Salmo trutta) from the New York State Rome Fish Hatchery. The age of brook trout used was 33-55 d post feeding (mean total length

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= 42 mm). Brown trout were 130-132 d post feeding (mean total length = 70 mm). The trout were not fed during the tests.

Testing was conducted in 20-L glass containers with 16 L of test solution. Rome spring water was used as the dilution water (pH = 7.61, hardness = 132 mg/L $CaCO_3$). Temperature was held at 9.4 $\pm$ 0.5°C using a thermostatically controlled water bath.

Stock solutions of each formulation were prepared by dissolving the insecticides in acetone to make a 10,000 μg/L resmethrin solution. These stock solutions were used to apply the pesticides to the test chambers. Test concentrations were 0.85, 1.2, 1.6, 2.3, 3.2, 4.5, 6.3, and 8.9 μg/L. Control containers were mixed with a volume of acetone equal to that of the highest resmethrin concentration.

Three types of toxicity tests were conducted with each formulation. All tests were conducted with 15 brook or 5 brown trout in each test chamber and 2 replica of each concentration. All tests were conducted twice. The first type of test was a series of static toxicity tests with both brook trout and brown trout. These tests were run for 96 hr with counts of mortality and intoxication taken every 24 hr. Dead fish were removed from the test containers and measured. We defined a fish as intoxicated if it was unable to remain upright or had jerky uncontrolled movements. Effective concentrations (EC) were based on the number of fish dead or intoxicated.

The second type of test was a short exposure/delayed effect test. Brook trout were placed into test solutions for 6 hr, removed, and placed into baskets held in flowing dilution water for 2 wk. These fish were monitored at 0, 1, 2, 4, 6, 18, 24 hr and daily thereafter. Counts of mortality and intoxication were taken at each time period.

The third type of test was a static toxicity test using brook trout and dilutions which had been mixed 24 and 48 hr prior to testing. These tests were conducted for 96 hr with counts taken every 24 hr.

The LC50s and EC50s were calculated using nominal concentrations and the probit or trimmed Spearman-Karber methods (Finney 1978; Hamilton et al. 1977). The LC50s calculated were used to compute a deactivation index. This deactivation index was determined by dividing the LC50 of the delayed exposure by the LC50 from the standard toxicity test (Marking 1972; Mauck et al. 1976). The index is equal to one when a pesticide is not deactivated with age, or greater than one when it is deactivated. In addition, we compared LC50s using the method in Sprague and Fogels (1977).

RESULTS AND DISCUSSION

Resmethrin was highly toxic to brook and brown trout in either formulation. Only small, statistically insignificant differences between the two formulations were observed during the 96-hr tests (Tables 1 and 2). The Scourge blank (PBO) was

Table 1. LC50s and EC50s (with 95% confidence limits) from standard and delayed exposure toxicity tests of synergized and non-synergized resmethrin to trout. The 72 hr and 96 hr LC50s were identical and are presented together. NA indicates that an EC50 was not calculated.

Species	Formulation	Time from preparation to test initiation, hr	Lethal/Effective Concentration (μg/L) (95% Confidence Interval)				
			24 hr		48 hr		72/96 hr
			LC50	EC50	LC50	EC50	LC50
Brown Trout	Synergized	0	1.74 (1.49-2.03)	1.00 (0.84-1.19)	1.35 (1.27-1.44)	NA -	1.31 (1.20-1.42)
Brook Trout	Synergized	0	3.07 (2.81-3.36)	1.35 (1.29-1.40)	1.92 (1.77-2.07)	1.16 (1.11-1.21)	1.83 (1.70-1.98)
		24	8.52 (6.51-15.8)	2.28 (2.02-2.55)	5.83 (4.94-7.20)	1.96 (1.66-2.32)	5.62 (4.80-6.78)
		48	6.47 (5.49-8.14)	2.14 (1.98-2.32)	4.91 (4.23-5.71)	NA -	4.91 (4.23-5.71)
	Non-synergized	0	3.26 (2.91-3.68)	1.38 (1.27-1.48)	1.78 (1.59-1.97)	1.32 (1.19-1.43)	1.69 (1.52-1.85)
		24	7.30 (5.87-11.2)	1.94 (1.52-2.26)	4.17 (3.43-5.10)	1.94 (1.52-2.26)	3.84 (3.30-4.46)
		48	7.07 (5.86-9.65)	3.47 (3.15-3.83)	4.01 (3.43-4.69)	3.04 (2.75-3.35)	3.71 (3.26-4.23)

found to be non-toxic in 96-hr tests. It did not kill any trout even at concentrations as high as 500 μg/L PBO. The PBO concentration in Scourge mixed to its 24-hr LC50 is only 9.21 μg/L. Thus, it is unlikely that PBO is directly toxic to trout.

Both formulations of resmethrin retained much of their toxicity, even when testing was initiated 48 hr after the test dilutions were mixed (Table 1). The mean 48-hr (delay) deactivation index was 2.23 for synergized resmethrin and 2.29 for non-synergized resmethrin, indicating that a similar deactivation and consequent reduction in toxicity had occurred in each formulation. Resmethrin in either formulation remained highly toxic to brook trout after "ageing" 48 hr. Mauck et al. (1976) determined a 7-d deactivation index of resmethrin to be about 2.0 (range 1.73-2.67) which is similar in magnitude to that determined in our study. Additional testing is needed to determine how long resmethrin remains toxic under field conditions.

Larger, older brown trout were significantly ($p < 0.05$) more sensitive to synergized resmethrin than younger brook trout especially during the first 24 hr of exposure (Table 2). A similar difference was found for the non-synergized formulation. The 24-hr LC50 for the non-synergized formulation of 1.18 μg/L for brown trout (from trimmed Spearman-Karber analysis of data in Skea et al. 1975) was significantly different ($p < 0.05$) than 3.26 μg/L for brook trout.

Table 2. Statistical significance of LC50 comparisons between trout species, formulations, and the amount of time in the delay of toxicity tests of resmethrin. Statistical significance ($p < 0.05$) is indicated by an S (significant), NS (nonsignificant), and NA (not available).

Test Conditions	Contrast	Length of Test		
		24 hr	48 hr	72/96 hr
Standard Test with Scourge	Brook vs Brown Trout	S	S	S
Standard Test with SBP-1382	Brook vs Brown Trout	S	NA	NA
Standard Test with Brook Trout	Scourge vs SBP-1382	NS	NS	NS
24-hr Delayed Test with Brook Trout	Scourge vs SBP-1382	NS	NS	NS
48-hr Delayed Test with Brook Trout	Scourge vs SBP-1382	NS	S	S
Scourge Test with Brook Trout	24-hr vs 48-hr Delayed	NS	NS	NS
SPB-1382 Test with Brook Trout	24-hr vs 48-hr Delayed	NS	NS	NS

Compared with the non-syngerized formulation, the synergized formulation clearly exhibits a greater intoxicating effect following the 6-hr exposure (Figure 1). Very little difference is seen between the two formulations following a 24-hr exposure. Trout which became intoxicated from either formulation recovered (within 4 d) or died (within 2 d) following the 6-hr exposure. No other delayed effects were apparent 4 to 14 d following the 6-hr exposures.

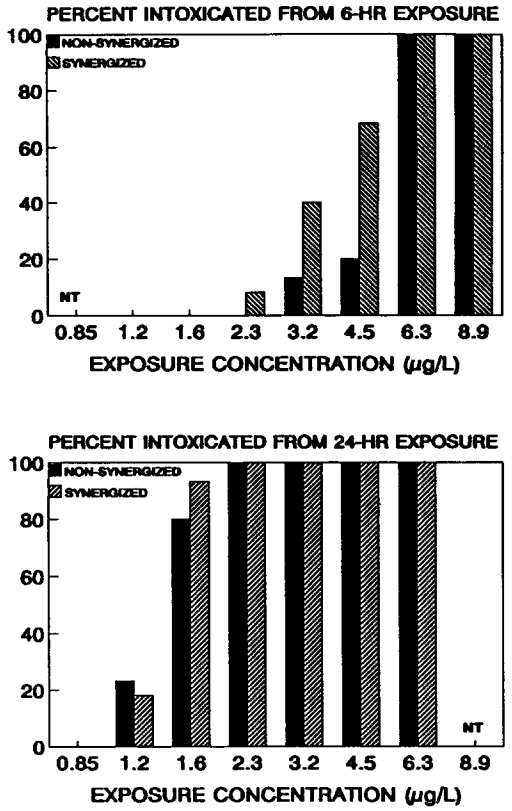

Figure 1. Percentage of brook trout intoxicated from exposure to synergized and non-synergized resmethrin after 6-hr (counted 24 hr after exposure) and 24-hr exposures. NT indicates that the concentration was not tested. N = 60 fish at each concentration.

Greater percentages of intoxicated brook trout eventually died at 24- and 48-hr intervals after the 6-hr exposure to the synergized formulation as compared with the non-synergized formulation. The results for the 6-hr exposure tests are in contrast with the tests for 24- and 48-hr continuous exposures (Figure 2). Continuous exposures to formulations for 24 and 48 hr resulted in little difference in mortality between synergized and non-synergized resmethrin, although these exposures resulted in higher mortality and mortality at lower concentrations as compared to the 6-hr exposures.

The results of our standard toxicity tests are similar to those of other investigators. Marking and Mauck (1975) report a 96-hr LC50 of 2.20 µg/L for rainbow trout (Oncorhynchus mykiss) to resmethrin. Mauck et al. (1976) obtained 96-hr LC50s of 0.450 µg/L, 2.62 µg/L and 2.36 µg/L for steelhead (O. mykiss), bluegill (Lepomis macrochirus), and yellow perch (Perca flavescens), respectively. Brook and brown trout seem to exhibit sensitivities to resmethrin which are similar to other species in static toxicity tests.

Lethal concentrations of PBO to fish are most likely to be in the mg/L range. Erickson et al. (1988) determined the 96-hr LC50 of PBO to rainbow trout to be

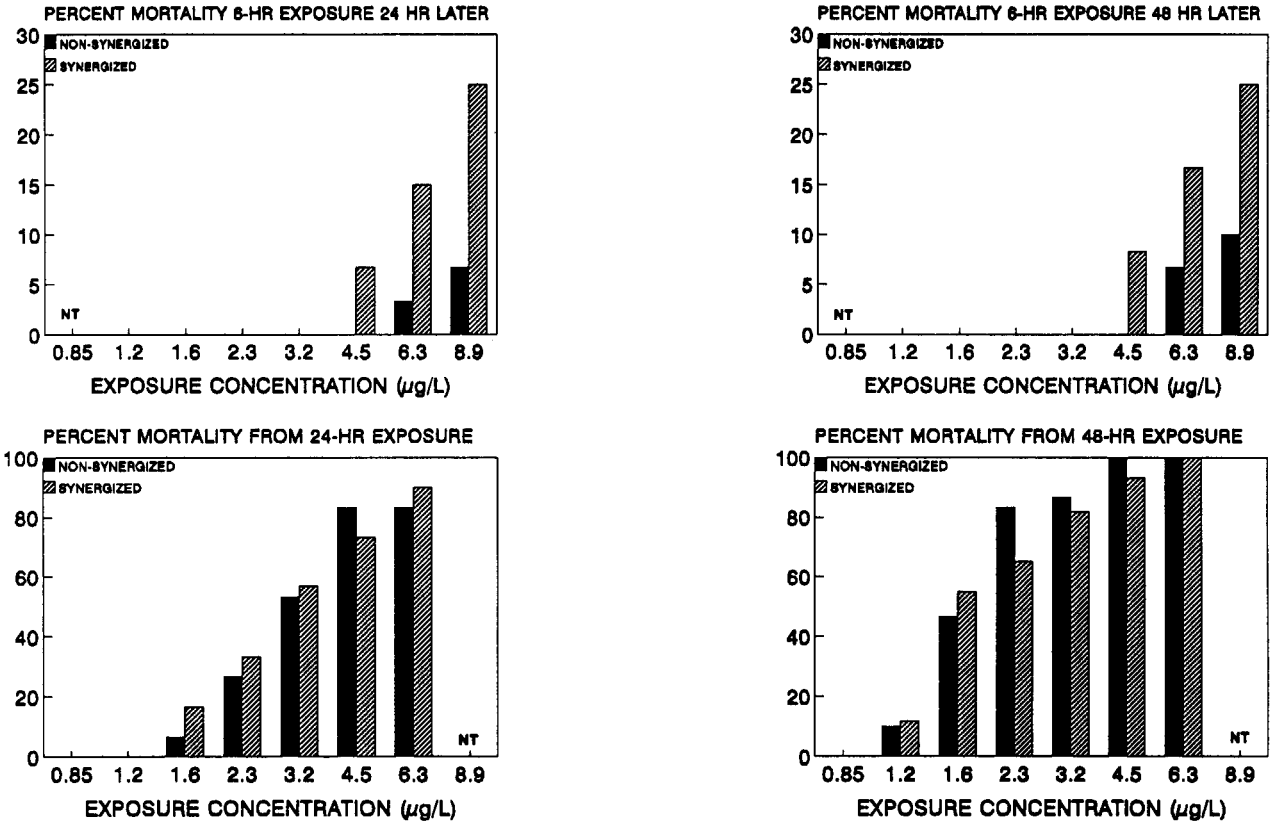

Figure 2. Percentage of brook trout which died from exposure to synergized and non-synergized resmethrin after 6-hr (counted 24 and 48 hr after exposure), 24-hr, and 48-hr exposures. NT indicates that the concentration was not tested. N = 60 fish at each concentration.

9800 μg/L and is consistent with our observation of no effect at 500 μg/L. Johnson and Finley (1980) report a 96-hr LC50 for PBO to rainbow trout of 3.4 μg/L and to bluegill of 4.2 μg/L, which is in sharp contrast to our study and Erickson et al. (1988). We might expect the toxicity of the synergized resmethrin insecticide to be much higher than that of the non-synergized resmethrin, due to a direct toxic effect of the PBO, if the LC50 of PBO were in the μg/L range.

The increase in the rate of intoxication due to short-term exposure to resmethrin synergized with PBO as compared to non-synergized resmethrin may be an important factor in evaluating the ecological impact of synergized resmethrin in aquatic systems. The addition of the synergist had a large effect on the toxicity of resmethrin following 6-hr exposures and increased the proportion of test trout which became intoxicated from a particular dose (Figure 1). In addition, trout exposed to the synergized formulation were less likely to recover following 6-hr exposures (Figure 2). Intoxicated fish were unable to maintain themselves against the slight current in their holding baskets. Many of these intoxicated trout recovered after 2 or 3 d. In the field it seems likely that trout intoxicated in this manner would be injured or subjected to increased predation pressure. Since there was no significant difference between the standard LC50s of the synergized and non-synergized formulations, researchers might be lead to the erroneous conclusion that the addition of the synergist has no effect on non-target aquatic organisms. Standard 24- to 96-hr toxicity tests failed to detect differences in the rate of intoxication between the synergized and non-synergized formulations which may be ecologically significant.

PBO may interfere with the detoxification of resmethrin, thereby increasing the percentage of fish intoxicated or killed in our 6-hr exposures to the synergized resmethrin. Reinbold and Metcalf (1976) found that PBO inhibited physiological processes which detoxified several organochlorine insecticides in green sunfish (Lepomis cyanellus). A much larger proportion of these insecticides remained in the form of the parent compound when PBO was present. Metcalf et al. (1974) found that PBO increased the toxicity of methoxychlor and trifluralin to green sunfish (also see Reinbold and Metcalf 1976). They postulated that the increased toxicity, which also extended the time over which the fish died, was due to the insecticides not being metabolized. In our toxicity tests 24 hr and longer, it appears that the uptake of resmethrin exceeds the ability of the fish to detoxify the pesticide. Demoute (1989) states that the high toxicity of pyrethroids to fish is due in part to a reduced ability to metabolize them. This may help to explain why the effect of the synergist is no longer seen during exposures longer than 24 hr.

Widespread black fly and mosquito control has taken place in the Adirondack Mountains in New York State over the last 30 years (Dean and Ford 1983). Several towns conduct spray programs that use Scourge as an adulticide (Ford 1990). The accidental spraying of a small headwater stream in the Adirondack region is likely to produce short, pulse type exposures to insecticides (Norris et al. 1983). The synergist (PBO) may exacerbate the toxicity of resmethrin to non-target organisms during short-term exposures as indicated in this study. Consequently, evaluations of the toxicity of

Scourge to non-target organisms based on studies of non-synergized formulations of resmethrin may underestimate the impact of Scourge on non-target organisms under field conditions.

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