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Toxicity of Fenvalerate and Its Constituent Isomers to the Fathead Minnow, *Pimephales promelas*, and Bluegill, *Lepomis macrochirus*

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The pyrethroid insecticides, including fenvalerate ([R,S]- ∝ -cyano-3-phenoxybenzyl [R,S]-2-[4-chlorophenyl] -3-methylbutyrate), are extremely toxic to fish. Factors such as pyrethroid and emulsifier interactions and pyrethroid stereochemistry may influence the toxicity of these insecticides to fish (Bradbury et al. 1985; Coats and O'Donnell-Jeffery 1979; Zitko et al. 1979). The stereochemical structure of pyrethroid insecticides also greatly influences their toxicity to insects and mammals. Fenvalerate, with two chiral centers (Figure 1), is a mixture of four stereoisomers. The present study further evaluates the influence of emulsifiers on the lethality and uptake of fenvalerate in fathead minnows (Pimephales promelas) and also provides initial information regarding the differential lethality of fenvalerate's stereoisomers in the fathead minnow and bluegill (Lepomis macrochirus).

MATERIALS AND METHODS

Quadruplicate static exposure tests using fathead minnows were run using 3.5-L glass aquaria, each containing 10 randomly assigned fish. Tests were run at 22°C with a 12:12 light:dark photoperiod and using carbon-filtered Ames, IA, municipal water. Dissolved oxygen, pH, alkalinity, hardness, and conductivity were determined (APHA 1980) at the initiation and completion of each test for water sampled from control chambers as well as from the highest and lowest toxicant concentrations. Overall means (± S.D.) were: hardness and alkalinity 165 ± 26 and 31.5 ± 8.8 mg/L as $CaCO_3$ (N = 108), respectively; pH 7.4 ± 0.4 (N = 100); dissolved oxygen 7.3 ± 2.1 mg/L (N = 110); and conductivity 486 ± 93 umho/cm (N = 110).

Technical-grade fenvalerate, a 30% a.i. (w/v) EC formulation, and the 2S,∝S; 2S,∝R; 2R,∝S; and 2R,∝R isomers (Figure 1) were

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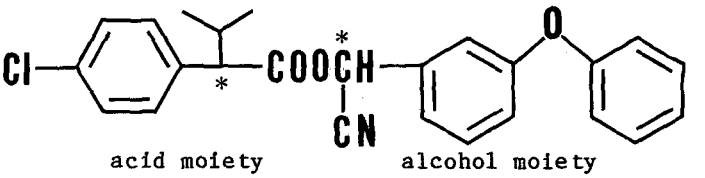

Figure 1. Chemical structure of fenvalerate denoting chiral centers (*). Either chiral carbon may be R or S, thereby yielding four possible enantiomers.

provided by Shell Development Company, Modesto, CA. Original plans were to test each of the four stereoisomers individually; however, preliminary studies showed that the α -hydrogen could exchange with available protons in certain solvents. To further test the potential for racemization, the 2S,α R isomer was placed in 25-mL opaque glass vials along with one of eight solvents. Subsamples (3 uL) were taken 0, 1, 2, 4, 8, 19, 27, and 45 h after solvent addition. Methanol, ethanol, dimethyl formamide, and dimethyl sulfoxide racemized the 2S,α R to the 2S,α S isomer; no racemization occurred with acetone, hexane, acetonitrile, or ethyl acetate (Table 1). When water was tested, variable amonts of racemization were noted; the degree of racemization seemed to be strongly dependent on the pH of the water tested. Racemization of the alcohol moiety was confirmed by chiral HPLC using a Pirkle phenylglycine column (Cole Parmer Instrument Co.) per method of Papadopoulou-Mourkidou (1985). This racemization, also reported by Hill (1981), made it impossible to test individual isomers via an aqueous exposure. Since the optical integrity of the acid moiety is unaffected in water, lethality of the acid-resolved mixtures of the isomers (2S,α R,S and 2R,α R,S) were tested by using fathead minnows.

Preliminary studies indicated that aqueous fenvalerate concentrations in a static system decreased following first-order kinetics, with stable levels attained approximately 48 h after addition to test chambers (linear portion of the decay curve). By placing the fathead minnows in the chambers 48 h after addition of fenvalerate, acceptably stable concentrations were maintained throughout the test period. The rapid initial drop in fenvalerate concentration was attributed to losses on glass (Sharom and Solomon 1981) and required that the applied nominal concentrations be about four times greater than the actual desired levels. Fenvalerate formulations were introduced directly into the test water by using microliter amounts of a concentrated stock in acetone. The 2R,α R,S and 2S,α R,S mixtures were made up 1:1 by using the proper individual isomers.

Fish, obtained from the Kloubec Fish Farm (West Amana, IA), were laboratory acclimated for 10 d before use. Fish were not fed 24 h preceding or during the 48-h exposure. A geometric series of five exposure concentrations and a control treatment were used. Mortality counts and behavior observations were recorded four times during the first 24 h of exposure and three times in the

Table 1. Racemization of the 2S,∝R fenvalerate isomers in various solvents

Solvent	Percentage of 2S,∝S isomer in 2S,∝R standards following solvent addition: Time (h) After Solvent Addition 0	1	2	4	8	19	27	45
Ethanol	0[a]	0	0	0	3 ±7	19 ±22	31 ±10	44 ±1
Methanol	0	0	2 ±5	4 ±9	24 ±7	38 ±6	46 ±3	47 ±3
Dimethyl sulfoxide	0	6 ±12	9 ±18	24 ±17	29 ±15	45 ±6	47 ±5	48 ±4
Dimethyl formamide	11 ±13	46 ±3	48 ±3	48 ±2	48 ±2	47 ±1	48 ±2	48 ±2

[a] Mean ± 1 S.D. (N = 4). Unracemized samples (0% 2S,∝S) were 99.6% ± 5.1% (N = 39) of the initial nominal 2S,∝R concentration. No racemization was noted in acetone, hexane, acetonitrile, or ethyl acetate through 45 h.

remaining 24 h. Death was defined as complete immobilization and failure to respond to gentle prodding. As mortality occurred, the fish were removed, rinsed with acetone three times to remove any adsorbed toxicant, weighed, and stored at -20°C. Any fish alive at the termination of the tests and the controls were sacrificed and handled in the same manner.

A computer Trimmed Spearman-Karber method (Hamilton et al. 1977) was used to calculate LC_{50} values. Mortality counts from quadruplicate chambers were combined before analysis. Because mortality was confined to the first 27 to 30 h of exposure, the average of the 0 and 24-h measured aqueous fenvalerate concentrations were used in the calculations; 48-h fenvalerate concentrations usually were slightly lower than the 24-h values and use of the 48-h measurements artificially lowered the LC_{50} values.

Each replicate concentration series was monitored at least once for fenvalerate during a test with sampling at 0, 24, and 48-h postexposure. The 48-h postexposure values were only used to confirm fenvalerate stability in test aquaria. Aqueous fenvalerate concentrations were determined after hexane extraction and quantitation by gas-liquid chromatography (GC) based on previously published methods (Bradbury et al. 1985). Spike recovery was 98.3 ± 7.6% (N = 21).

To determine the whole-body residue levels, fathead minnows

exposed to various fenvalerate preparations were examined by Bradbury and Coats (1982). Fish were pooled and analyzed by chamber, thereby providing four replicate samples per exposure concentration. Fish alive at the termination of the test were analyzed separately from those that died. Extraction and cleanup of spiked fathead minnow samples resulted in 97.7 $\pm$ 5.8% (N = 9) recovery.

Lethality testing of individual fenvalerate enantiomers, via aqueous exposures, was not possible due to the exchange of the α proton and resulting racemization. To evaluate enantiomer toxicity, bluegill (44.1 $\pm$ 2.5 g) were exposed via an intraperitoneal (i.p.) injection to different isomers by using a vehicle (0.1 ml/10 g body weight) of ethanol:Emulphor-620 (GAF Corp., New York, NY):0.6% NaCl (1:1:8) (adapted from Glickman et al. 1981). Fish injected with vehicle alone served as controls. Stability studies (based on GC) indicated no significant occurrence of enantiomer racemization in the vehicle when used within 1.5 h. Ten fish were used for each of five exposure levels plus a control treatment. After dosing, fish were held in individual compartments within 55-L aquaria (five fish per aquarium, each aquarium fitted with an aerating charcoal filter and galvanized-steel mesh dividers) for 48 h. Mortality counts and behavior observations were made four times during the first 24 h after exposure and three times thereafter. Median LD_{50} values were calculated by using the Trimmed Spearman-Karber method (Hamilton et al. 1977).

RESULTS AND DISCUSSION

Technical and EC fenvalerate formulations were extremely toxic to fathead minnows; 48-h LC_{50} values were 1.13 and 0.93 ug/L, respectively (Table 2). The incipient lethalities of the two formulations were the same (overlapping 95% confidence intervals (CI)); during the first 24 h, however, the EC formulation was more toxic (numerically but not statistically)(Table 2). The technical formulation was tested a second time, at the end of the study, to provide a measure of quality control. Results of this test were not significantly different from the results initially obtained (48-h LC_{50} of 0.87 ug/L, 95% CI of 0.78 to 0.98 ug/L). The toxicities of the two isomer mixtures were markedly different from the technical material (Table 2). The 2S, α R,S isomer mixture was 3.3 times more toxic to fathead minnows than the technical formulation, with a 48-h LC_{50} of 0.34 ug/L. The 2R, α R,S isomer mixture was significantly less toxic to fathead minnows (48-h LC_{50} > 140 ug/L).

Whole-body concentrations of fenvalerate gradually increased with increasing exposure level, with mortality beginning at the following body concentrations: technical 800 ng/g; EC 400 ng/g; 2S, αR,S 200 ng/g. Concentrations above these levels were associated with mortality. No mortality occurred at the highest residue concentration (7320 ng/g) for 2R, α R,S.

Table 2. Acute toxicity of fenvalerate formulations and isomer mixtures to fathead minnows

Formulation or isomer mixture	LC_{50} (ug/L) with 95% confidence interval		Relative potency
	24 h	48 h	
Technical formulation (2R,S, ∝R,S)	1.69 (1.44-1.97)	1.13 (0.96-1.32)	1.0
30% EC formulation	1.06 (0.97-1.17)	0.93 (0.83-1.05)	1.2
2S, ∝R,S mixture	0.38 (0.32-0.45)	0.34 (0.28-0.40)	3.3
2R, ∝R,S mixture	>140	>140	<0.008

Time to mortality (Table 3) was generally consistent among preparations, with the most lethal concentrations eliciting mortality within 10 to 15 h. At the lowest lethal concentrations mortality occurred at about 30 h postexposure. Net uptake rates (Table 3), which are a function of uptake and excretion, increased at the higher aqueous exposure concentrations and shorter exposure periods. At comparable lethal aqueous exposure concentrations, uptake rates and residues were slightly greater (about 1.2 to 2 times) in the EC-formulation-exposed fish. This pattern is consistent with the slightly greater (but not statistically significant) toxicity of the EC formulation.

Of the four isomers tested with bluegill, only the 2S, ∝S isomer was more toxic than the technical formulation (Table 4). The 2S, ∝S isomer had a relative potency almost 100 times greater than the next most toxic isomer (2S, ∝R). Because of solubility limitations and trace isomer cross-contamination, 2.60×10^{5} ng/g and 2.12×10^{5} ng/g were the highest practical doses for the 2R, ∝R and 2R, ∝S isomers, respectively, and no mortality occurred at these levels. The 48-h LD_{50} values for the technical material, 2S, ∝S and 2S, ∝R were 670, 120, and 11600 ng/g, respectively.

The LC_{50} values reported here for technical and EC formulations of fenvalerate are similar to those reported by Bradbury et al. (1985) after flow-through exposures. The net uptake rates and times to mortality were very similar for both formulations and also were in agreement with previous results (Bradbury et al. 1985).

Results of the present study indicated no significant difference between lethalities of technical and EC formulations to fathead minnows, which is consistent with the findings of Bradbury et al. (1985). Coats and O'Donnell-Jeffery (1979) and Zitko et al. (1979), however, reported that EC formulations of several

Table 3. Fenvalerate whole-body residues, time to mortality, and net uptake rates for the formulations used in the acute toxicity tests with fathead minnows

Formulation	Exposure level(ug/L)[a]	Residue conc.(ng/g)[b]	Time to mortality(h)	Net uptake rate(ng/g/h)[c]
Technical	0.31(S)[d]	280 ± 173	-	6 ± 3[e]
	0.61(S)	430 ± 138	-	9 ± 3
	1.23(S)	800 ± 485	-	17 ± 7
	1.23(D)	830 ± 156	29 ± 7	29 ± 5
	2.29(D)	1160 ± 138	23 ± 9	53 ± 13
	3.98(D)	1220 ± 104	17 ± 4	75 ± 16
EC	0.33(S)	200 ± 71	-	4 ± 2
	0.70(S)	340 ± 71	-	7 ± 1
	0.70(D)	530 ± 184	27 ± 8	22 ± 10
	1.83(D)	900 ± 156	18 ± 7	51 ± 9
	3.69(D)	1140 ± 410	12 ± 7	94 ± 31
	6.20(D)	1350 ± 467	9 ± 6	144 ± 31
2S, αR,S	0.06(S)	90 ± 99	-	2 ± 1
	0.09(S)	230 ± 283	-	5 ± 4
	0.20(S)	360 ± 651	-	7 ± 8
	0.36(S)	400 ± 580	-	8 ± 7
	0.09(D)	230 ± 269	32 ± 6	6 ± 3
	0.20(D)	760 ± 891	24 ± 3	28 ± 18
	0.36(D)	1000 ± 849	24 ± 9	44 ± 24
	0.90(D)	1830 ± 2120	14 ± 6	197 ± 96
2R, αR,S	7.85(S)	2350 ± 692	-	49 ± 12
	12.7 (S)	3840 ± 745	-	80 ± 14
	32.7 (S)	5280 ± 1524	-	110 ± 27
	98.1 (S)	6260 ± 1126	-	130 ± 20
	137 (S)	7320 ± 1645	-	152 ± 30

[a] Average of the 0-h and 24-h postexposure water concentrations; toxicant was not detectable in control water (<0.01 ug/L).

[b] Reported on whole-body wet-weight basis, not corrected for recovery. Toxicant was not detectable in control fish (<4 ng/g).

[c] Uptake rate = residue concentration/time of exposure. For surviving fish, time of exposure was 48 h.

[d] Values obtained from samples derived from fish surviving exposure are denoted by (S); those samples derived from fish that died are denoted by (D).

[e] Values are means ± 1 S.D. (N = 4).

pyrethroid insecticides were two to nine times more toxic to rainbow trout (Salmo gairdneri) and Atlantic salmon (Salmo salar) than technical material (static tests with nominal or mathematically estimated toxicant levels). The differences in lethal response noted between these two studies and ours is likely a function of methodology. In the static studies of Coats and O'Donnell-Jeffery (1979) and Zitko et al. (1979), the nominal or initial toxicant concentrations required to elicit mortality were likely higher with the technical materials since emulsifiers tend to enhance the availability of active materials in EC formulations. These differences in exposure levels would artificially give the appearence of greater EC formulation

Table 4. Acute lethality of fenvalerate and its constituent isomers to bluegill

Isomer	48-h i.p. LD_{50} (ng/g) and 95% confidence interval	Relative potency[a]
2R,S, ∝R,S (Technical)	670 (520 - 860)	1.0
2S, ∝S	120 (100 - 140)	5.6
2S, ∝R	11600 (3830 - 35100)	0.06
2R, ∝S	$>2.12 \times 10^5$	<0.003
2R, ∝R	$>2.60 \times 10^5$	<0.003

toxicity. In our study, the insecticide was allowed to equilibrate in the exposure system before testing, thereby minimizing the confounding factors of the earlier static tests. Increased toxicity of the EC formulations in the previous studies (Coats and O'Donnell-Jeffery 1979; Zitko et al. 1979) could have been due to additive toxicity or synergism; however, based on residue analysis, synergistic effects between the commercial emulsifiers and fenvalerate are unlikely (Bradbury et al. 1985, and this study).

Testing of fenvalerate isomer mixtures indicated that fenvalerate toxicity is strongly dependent on stereochemical structure, with the lethality to fish primarily dependent on the 2S, ∝S component of the technical mixture. Results from aqueous exposures to fathead minnows and subsequent residue analyses indicated that an S configuration at the chiral carbon in the acid moiety was crucial for toxicity. The 2S, ∝R,S isomer was about two to three times more toxic than the technical material in comparisons of either aqueous exposure concentrations or whole body residues at mortality. Fenvalerate esters with an R configuration in the acid moiety were essentially nontoxic to fathead minnows.

Intraperitoneal testing in bluegill indicated that an S orientation at the ∝-carbon in the alcohol moiety also imparts greater toxicity to the ester structure. The 2S, ∝S isomer was 5.6 times more toxic than the technical material, whereas the 2S, ∝R isomer was 17 times less toxic than the technical material. It is unknown at this time whether the toxicity noted in the 2S, ∝R isomer was due to actual toxicity, contamination by the 2S, ∝S isomer in the test solutions, or in vivo racemization to the 2S, ∝S isomer. An R configuration in the acid moiety also dramatically reduced the lethality of the ester to bluegills. These trends between stereochemical structure and lethality in fish are similar to those published for insects and mammals (Nakayama et al. 1979).

The i.p. LD_{50} of 670 ng/g (0.67 mg/kg) for bluegill and whole-body residues at mortality of 800 ng/g (0.8 mg/kg) for fathead minnows reported in this study clearly indicate that fish are much more sensitive to fenvalerate than are birds and mammals. Soderlund and Casida (1977) reported a mouse i.p. LD_{50} of >500 mg/kg, whereas Nakayama et al. (1979) and Shell (1975) report rat and mouse oral LD_{50} values of 450 mg/kg and 245 mg/kg, respectively. Bradbury and Coats (1982) reported an acute oral LD_{50} of >4000 mg/kg for bobwhite quail (Colinus virginianus). Possible contributing factors in the extreme susceptibility of fish to fenvalerate include inefficient metabolism (Bradbury et al. 1986), and sensitivity at the site(s) of action.

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