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Keywords
Fenvalerate, Emulsifiable concentrate, Pimephales promelas, Uptake, Acute toxicity

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DIFFERENTIAL TOXICITY AND UPTAKE OF TWO FENVALERATE FORMULATIONS IN FATHEAD MINNOWS (*PIMEPHALES PROMELAS*)

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Abstract—The influence of the commercial emulsifier on the acute toxicity and uptake of fenvalerate [(*R*,*S*)-α-cyano-3-phenoxybenzyl(*R*,*S*)-2-(4-chlorophenyl)-3-methylbutyrate], a synthetic pyrethroid insecticide, by fathead minnows (*Pimephales promelas*) was examined. Flow-through acute toxicity testing with measured concentrations of technical-grade fenvalerate and a 30% active ingredient emulsifiable concentrate (EC) of the insecticide was conducted. Steady-state LC50 values were reached by 72 and 120 to 168 h, respectively, in tests with technical-grade and EC formulations of this insecticide. Initially, technical-grade fenvalerate was more toxic; the 96-h LC50s for technical-grade fenvalerate and the EC were 0.69 and 0.99 μg/L, respectively. By 168 h, an LC50 of 0.75 μg/L was determined for the EC, indicating that the incipient lethalities of the two formulations were similar. Fenvalerate concentration factors and residue levels in fish showed no significant differences between formulations. Residue levels associated with mortality decreased slightly with increasing fenvalerate water concentrations and ranged (mean) from approximately 1,000 to 1,500 ng/g. Levels in fish that survived testing increased with increasing exposure concentrations and ranged from about 600 to 900 ng/g. Concentration factors of 187 to 1,860 were calculated, with a mean of 1,670, as determined from fish that survived testing. The time required to accumulate residues was greater with the EC and contributed to a significantly slower uptake rate. Slower fenvalerate uptake in the presence of emulsifiers seemingly resulted in the initially lower toxicity of the EC formulation.

Keywords—Fenvalerate Emulsifiable concentrate *Pimephales promelas* Uptake Acute toxicity

INTRODUCTION

The synthetic pyrethroid insecticides are very toxic to many aquatic species [1]. Some reports also indicate that emulsifiers may enhance the toxicity of pyrethroids to fish. Based on static 24-h LC50 tests and nominal water concentrations, Coats and O'Donnell-Jeffery [2] found that emulsifiable concentrate (EC) formulations of permethrin, fenvalerate, cypermethrin and fenpropanate were 2.2, 3.6, 5.0 and 8.9 times, respectively, more toxic to rainbow trout (*Salmo gairdneri*) than were the corresponding technical-grade materials. Zitko et al. [3], examining Atlantic salmon (*Salmo salar*), reported static 96-h lethal thresholds of 0.59 and 1.97 μg/L for Decis®, a 25% active ingredient (w/v) EC of deltamethrin, and technical-grade deltamethrin, respectively. Increased fish mortality has also been reported with several other formulated pesticides, e.g., endosulfan [4,5] and aminocarb [6]. Various ubiquitous surfactants, including detergents, have been reported to occasionally influence the toxicity of other pesticides as well [7,8].

Although previous work [2,3] implies increased lethality of pyrethroids because of the presence of emulsifiers, several techniques in the methodologies, including static exposures, nominal or mathematically estimated toxicant concentrations and the use of solvent carriers in testing technical materials, may have biased the results. Seemingly increased lethality of pyrethroid EC formulations could result from (a) additive toxicity, (b) synergism and/or (c) enhanced uptake of the active ingredient (possibly due to increased availability).

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At this time, however, no specific mechanism has been proposed and examined.

The research presented in this report is an initial phase of a project designed to determine the influence of emulsifiers on the toxicity of pyrethroid insecticides to fish. Fenvalerate is the model compound and the fathead minnow (*Pimephales promelas*) is the test species. More specifically, the investigation assesses the acute toxicity and residue accumulation of technical-grade fenvalerate and a commercial 30% a.i. (w/v) EC formulation under flow-through conditions and with no solvent carriers.

MATERIALS AND METHODS

Exposure system

Flow-through tests were conducted at the U.S. Environmental Protection Agency (EPA) Environmental Research Laboratory-Duluth, Duluth, Minnesota, in an enclosed diluter system modeled after that of Benoit et al. [9]. The system delivered five toxicant concentrations and control water to quadruplicate glass test chambers ($19 \times 7.5 \times 10$ cm, filled to a depth of 7.5 cm, with a tank volume of 0.7 liter) at a flow rate of 15 ml/min. A 16-h photoperiod was used, with light produced by fluorescent bulbs at an intensity of approximately 250 lux.

Unfiltered Lake Superior water (24.7 ± 0.2°C) was used throughout the testing. Dissolved oxygen (DO), pH, alkalinity and hardness were determined daily [10] for water sampled from control chambers as well as from chambers of the highest and lowest fenvalerate concentrations. Overall means for hardness and alkalinity were 45.8 (range 44.3 to 47.3) and 42.7 (range 41.1 to 44.5) mg/L as $CaCO_3$, respectively. The pH ranged from 7.3 to 7.8. Mean DO was 7.6 mg/L (range 7.2 to 8.0).

Toxicant preparation

Technical-grade fenvalerate (lot number 80115, 93% purity), a 30% a.i. (w/v) EC formulation and the placebo EC (an EC formulation without fenvalerate) were provided by the Shell Development Company, Modesto, California.

Technical-grade fenvalerate was put into solution using five glass column saturators (unpublished method, D. Defoe, U.S. EPA Environmental Research Laboratory-Duluth, Duluth, MN). Five glass columns (1.3 m $\times$ 22 mm i.d.) were packed with glass wool and, under vacuum, a solution of fenvalerate in acetone (17 g/250 ml, approx. 50 ml per column) was evenly applied to each column. After evaporation of the acetone, the columns were connected in line with Teflon-lined tubing. Lake Superior water was pumped through the columns and delivered to the toxicant cell of the diluter at a flow rate of 115 ml/min with an FMI chemical metering pump (Fluid Metering, Inc., Oster Bay, NY). The concentrations of fenvalerate in the system were chemically analyzed daily for 7 d before testing to ensure that concentrations were stable.

The EC formulation was introduced by preparing a stock emulsion of the 30% formulation in an 18-liter glass stock bottle (nominal concentration of 1.5 mg/L). The emulsion was stirred throughout the test period with a Teflon-coated magnetic stir bar to ensure a uniform distribution of the insecticide. The emulsion was delivered to the toxicant cell at a flow rate of 1 ml/min with an FMI pump. The fenvalerate concentrations in the system were chemically analyzed daily for 3 d before testing.

Water analysis

Each of the four replicate concentration series was monitored once during the course of a test. Four-hundred fifty milliliters of water, collected at middepth in a chamber, were vigorously stirred with 50 ml of pesticide-grade hexane in a 500-ml volumetric flask for 1 h. Concentrations of fenvalerate in the hexane extract were determined using a Hewlett-Packard 5710A gas chromatograph equipped with a ^{63}Ni electron-capture detector and a coiled glass column (1.0 m $\times$ 1 mm i.d.) containing 3% OV-7 on 80/100 mesh Chromosorb W-HP (Anspec Co., Inc., Ann Arbor, MI). Argon with 5% methane was used as a carrier gas at a flow rate of 34 ml/min. Injector and detector temperatures were 250 and 300°C, respectively. The column oven was operated at a temperature program mode set at a rate of 4°C/min, with initial (4 min) and final temperatures of 220 and 250°C, respectively, adapted from the method of Holcombe et al. [11]. Fenvalerate eluted as two peaks (retention times of 7.2 and 7.4 min), each corresponding to a pair of its enantiomers. Standard curves based on the sums of peak areas were used for quantitation. Extraction of water spiked with technical-grade fenvalerate and the EC resulted in 103 ± 6 ($n = 4$) and 100 ± 4% ($n = 4$) recovery, respectively.

Biological procedures

Fathead minnows, 30 to 31 d old and obtained from the stock culture of the U.S. EPA Environ-

mental Research Laboratory-Duluth, were used. Control fish at the termination of testing weighed 83.5 ± 12.8 mg.

Initially, a 96-h static screening test was performed using the placebo EC. Nominal concentrations of 0, 20, 200, 2,000 and 10,000 μg/L were used in duplicate chambers. Five fish were placed in each chamber. No mortality or signs of intoxication were noted.

In the flow-through tests, groups of 10 fish were randomly assigned to each of the 24 chambers. Fish were not fed for 24 h before testing or during the first 4 d of each test. From 96 through 168 h, the remaining fish in the EC test were fed two to three frozen brine shrimp per fish three times daily. Uneaten shrimp were removed 1.5 h after being placed in the chambers.

During testing, fish were observed four times during the first 24 h and then three times daily during the remaining exposure period. The time to death for each fish was recorded. Death was defined as complete immobilization and failure of the fish to respond to gentle prodding. Signs of intoxication were noted by comparing the responses of fenvalerate-exposed fish with those of the controls. Dead fish were removed, rinsed three successive times in acetone (to remove adsorbed fenvalerate), weighed and then stored at −20°C. At test termination, controls and surviving fenvalerate-exposed fish were handled as described, except that control fish were not rinsed with acetone.

A computer Trimmed Spearman-Karber method [12] was used to calculate LC50 values. Mortality counts from quadruplicate chambers were combined before analysis.

Residue analysis

Fish (either all survivors or all dead) from the various dose groups were pooled and analyzed by chamber, thereby providing four replicate samples per formulation exposure concentration. Survivors from the technical-grade 0.75 μg/L fenvalerate concentration were pooled as one sample. Survivors from the technical-grade 1.33 μg/L fenvalerate and EC 0.92 μg/L fenvalerate exposures and dead from the technical-grade 0.49 μg/L fenvalerate concentration were not analyzed because of insufficient numbers. Analysis of the fish was based on methods previously described for quail tissue [13]. Extraction and cleanup of spiked fathead minnow samples resulted in 99.5 ± 2.7% ($n = 4$) recovery.

Statistical analysis

To describe fenvalerate uptake by fathead minnows in terms of water concentration and to determine any effects of the emulsifier, analysis of covariance [14] was performed on the residue, concentration factor (CF), time to mortality and uptake rate data. Before analysis, treatment variances were tested for homogeneity using Bartlett's test [14] and, when required, data were log-transformed. A p value of 0.05 was used to determine significance.

RESULTS

Fenvalerate was extremely toxic to fathead minnows. Median lethal concentrations for both formulations are plotted in Figure 1. The LC50 values for both formulations initially decreased over time. An LC50 of 0.69 μg/L for technical-grade fenvalerate was reached at 72 h and was unchanged through 96 h, at which time the test was terminated. The LC50 values obtained from the EC test continued to decline after 96 h (96-h LC50 0.99 μg/L) and reached an apparent steady state between 120 and 168 h, with a 168-h LC50 of 0.75 μg/L. In both tests, a very steep concentration-response curve was noted between approximately 0.9 and 0.6 μg/L. Comparison of LC50 values through 96 h (Table 1) indicates that the technical-grade material was 1.8 to 1.4 times more toxic than the EC. From 144 to 168 h, the EC LC50 values were comparable with the technical-grade fenvalerate 96-h LC50 (overlapping 95% confidence levels), which suggests that the incipient lethal concentrations of the two formulations to fathead minnows were essentially the same.

Signs of intoxication were monitored and found to be similar for both formulations. Initially,

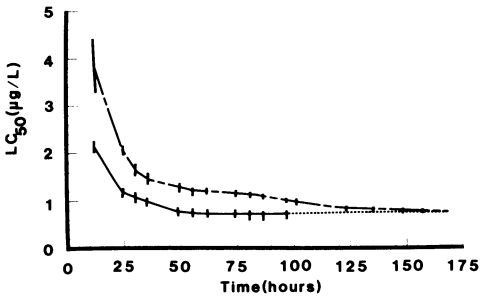

Fig. 1. Lethality of the technical-grade (solid line) and emulsifiable concentrate (dashed line) formulations of fenvalerate to fathead minnows over time. Bars represent 95% confidence intervals.

Table 1. Acute toxicity of two fenvalerate formulations to fathead minnows

Formulation	LC50 (μg/L) and 95% confidence interval[a]						
	24-h	48-h	72-h	96-h	120-h	144-h	168-h
Technical-grade	1.14 (1.26–1.02)	0.77 (0.86–0.70)	0.69 (0.74–0.63)	0.69 (0.74–0.63)	—	—	—
30% emulsifiable concentrate	2.06 (2.18–1.94)	1.31 (1.42–1.21)	1.16 (1.26–1.07)	0.99 (1.08–0.92)	0.83 (0.89–0.78)	0.78 (0.82–0.74)	0.75 (0.79–0.73)

[a]Based on combined mortality data from replicate exposure chambers.

intoxicated fish were observed to swim near the surface of the water; this behavior was followed by general hyperactivity and darting, which progressed to erratic bursts of swimming activity and culminated in episodes of violent, whole-body contractions. After a spastic outburst, the affected fish were inactive, lying on the bottom of the chamber until the next episode. Once a fish reached this level of intoxication, mortality eventually resulted. Within each test, the time to initial intoxication increased at lower fenvalerate concentrations; however, fish at technical-grade fenvalerate concentrations comparable with those in the EC test showed signs of intoxication sooner. Fish in the highest concentration of each test showed signs of toxicity within 1 h. By 36 h, all fish, except those exposed to the lowest concentrations, showed signs of toxicity. Fish exposed to 0.37 and 0.58 μg/L fenvalerate in the technical-grade and EC tests, respectively, never seemed intoxicated.

Results of the residue analysis are presented in Table 2. Residues at comparable fenvalerate exposure concentrations were similar between the formulations, with mean values ranging from 598 to 1,510 ng/g. Fenvalerate concentrations in dead fish ranged from 1,010 to 1,510 ng/g, whereas mean concentrations in fish that survived exposure ranged from 598 to 911 ng/g. Fish from the EC 8.11 μg/L fenvalerate concentration had residue concentrations markedly higher than those from the EC 3.22 μg/L group; this was not consistent with the trends observed in the other lethal expo-

Table 2. Fenvalerate whole-body residues,[a] time to mortality, uptake rates[b] and concentration factors (CFs) for the two fenvalerate formulations used in the acute toxicity tests with fathead minnows

Formulation	Mean water concn. (μg/L)	Residue concn. (ng/g)	Time to mortality (h)	Net uptake rate (ng/g/h)	CF
Technical-grade	ND (S)	ND	—	—	0 ± 0
	0.37 ± 0.13(S)[c]	598 ± 124	—	—	1,620 ± 335
	0.49 ± 0.12(S)	911 ± 147	—	—	1,860 ± 301
	0.75 ± 0.11(S)[d]	1,680	—	—	2,240
	0.75 ± 0.11(D)	1,220 ± 122	45 ± 10	28 ± 7	1,620 ± 162
	1.33 ± 0.10(D)	1,050 ± 79	25 ± 2	39 ± 6	791 ± 60
	3.51 ± 0.41(D)	1,010 ± 109	11 ± 1	93 ± 14	288 ± 31
30% emulsifiable concentrate	ND (S)	ND	—	—	0 ± 0
	0.58 ± 0.03(S)	887 ± 76	—	—	1,530 ± 130
	0.92 ± 0.05(D)	1,300 ± 99	107 ± 5	12 ± 1	1,410 ± 107
	1.49 ± 0.13(D)	1,170 ± 64	42 ± 7	28 ± 6	784 ± 43
	3.22 ± 0.14(D)	1,030 ± 89	18 ± 3	53 ± 9	321 ± 28
	8.11 ± 0.59(D)[d]	1,510 ± 145	11 ± 2	134 ± 15	187 ± 3

ND, not detectable (<0.01 μg/L in water, <0.01 ng/g in tissue).
[a]Reported on a whole-body wet-weight basis, not corrected for recovery.
[b]Uptake rate = residue concentration/time to mortality.
[c]Water concentrations based on active ingredient. Values are means ± standard deviation ($N = 4$, except for technical-grade 0.75(S) residue concentration, where $N = 1$, and emulsifiable concentrate 1.49(D) residue concentration where $N = 3$). Values obtained from samples derived from fish surviving fenvalerate exposure are denoted by (S); those samples derived from fish which died are denoted by (D).
[d]Data from these exposure concentrations were not used in statistical analyses; see the text.

sures (decreasing residues with increasing fenvalerate water concentrations). These higher residue concentrations could be an abberation or may suggest a more complex response. Because of the geometric selection of the exposure concentrations and the consequent lack of data between 3 and 8 μg/L, it is difficult to attribute these results to either possibility. Because of the uncertainty regarding the EC 8.11 μg/L residue data and the lack of a comparable exposure group in the technical-grade test, making its use in covariant analysis tenuous, this information was not included in any of the following statistical analyses. Pooled survivors from the technical-grade 0.75 μg/L fenvalerate groups were also not included in analyses because of the lack of replication.

Analysis of covariance was performed on residue data for surviving fish and dead fish. Fenvalerate concentrations in surviving fish included those of the controls and fish exposed to lethal fenvalerate exposures up to and including 0.75 and 0.92 μg/L from the technical-grade and EC tests, respectively. These data described residue accumulation in fish exposed to nonlethal fenvalerate concentrations through concentrations approximating a lethal threshold. Concentrations in dead fish exposed to 0.75 to 3.51 (technical-grade) and 0.92 to 3.22 (EC) μg/L fenvalerate were analyzed statistically to characterize uptake in fish exposed to lethal concentrations. In surviving fish, the rates of increase in fenvalerate residues with the increase in water concentration (Fig. 2) for the different formulations were not significantly different and could be characterized by a common slope. The concentration of fenvalerate in fish exposed to the two formulations, at nonlethal concentrations, could be described by: residue concentration = 70 + 1,520 (water concentration).

Analysis of covariance for concentrations of fenvalerate in dead fish also did not show a statistically significant effect of the two formulations. No difference between slopes was detected. The common slope was significantly different from 0 ($t_{20} = 15.68$, $p = 0.0008$). The resulting common curve (Fig. 2) for both formulations is as follows: residue concentration = 1,240 − 80 (water concentration).

Fenvalerate CFs (fish residue concentration divided by mean water concentration) determined from the residue data provided another means of

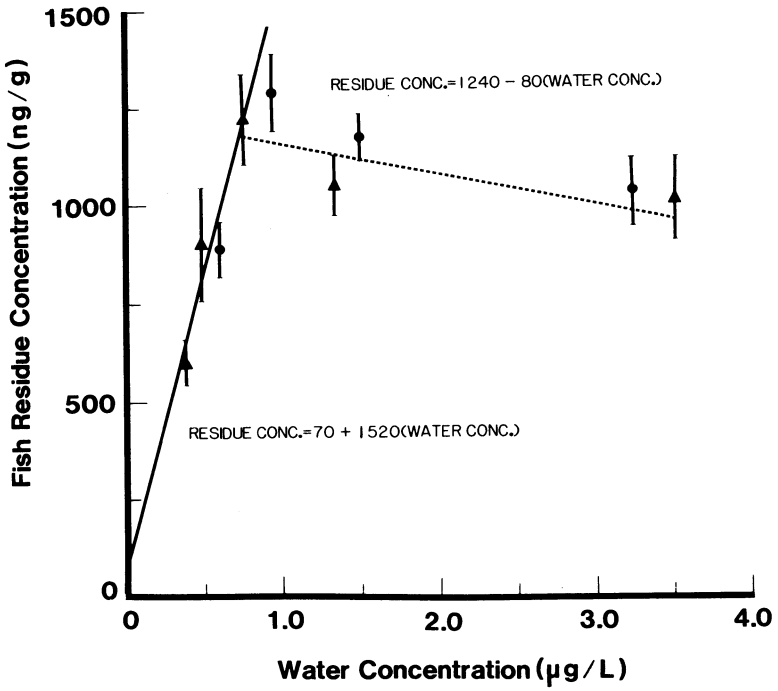

Fig. 2. Relationship between fish residue concentrations and lethal (dashed line) or sublethal to lethal (solid line) fenvalerate water concentrations for both technical-grade (triangles) and emulsifiable concentrate (circles) formulations. Bars represent ±1 SD.

describing the uptake of fenvalerate (Table 2). At comparable exposure concentrations, similar values were determined for each formulation, with higher CFs resulting from lower fenvalerate water concentrations (mean CFs ranged from 187 to 1,860). A log transformation of the CF data was required to eliminate inequality of the variances. A plot of log CF versus log fenvalerate water concentration (Fig. 3) suggested a linear response, with some evidence of a plateau at the lower fenvalerate water concentrations. Analysis of covariance indicated that there was no significant difference between the responses of the two formulations. A common curve of log(CF) = 2.97 – 0.87 log(water concentration) described the observed relationship.

Although the incipient lethal concentrations, residues and CFs were not significantly different between the two formulations, the time to mortality for fish, at comparable exposure concentrations, was markedly shorter with the technical-grade material (Table 2). In both tests, mean time to death was inversely related to fenvalerate water concentration. The time to mortality with the EC formulation was longer at all concentrations than that for comparable concentrations of technical-grade fenvalerate. Mean times to mortality in the test with technical-grade fenvalerate ranged from 11 to 45 h, whereas in the EC test, at comparable exposure concentrations, mean times to mortality ranged from 18 to 107 h. Analysis of covariance with log-transformed data (to stabilize variances) indicated that the relationships between time to death and fenvalerate water concentration for the two formulations were significantly different. Equations for the curves for the two formulations are given in Figure 4.

Since the time required to accumulate lethal residues was longer with the EC formulation, the net uptake rate of fenvalerate was slower in the presence of the emulsifier(s). Table 2 lists mean uptake rates for those fish that died during testing. Analysis of covariance using a log transformation of uptake rates (to eliminate unequal variances) and log fenvalerate water concentrations confirmed that at comparable exposure concentrations the uptake rate of technical-grade fenvalerate was significantly greater.

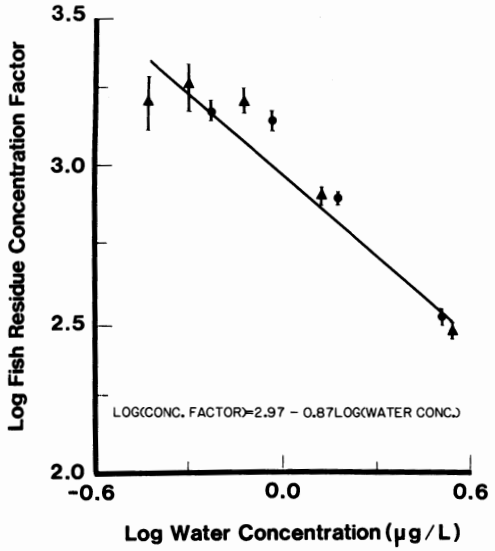

Fig. 3. Relationship between the log fish residue concentration factor and log fenvalerate water concentration for technical-grade (triangles) and emulsifiable concentrate (circles) formulations. Bars represent ±1 SD. Concentration factors associated with the lowest three water concentrations were derived from data for fish that survived lethality testing.

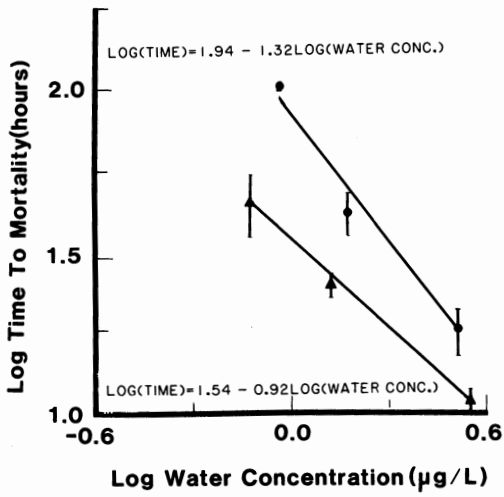

Fig. 4. Relationship between log time to mortality and log fenvalerate water concentration for fish exposed to technical-grade (triangles) or emulsifiable concentrate (circles) formulations. Bars represent ±1 SD.

DISCUSSION

The LC50 values determined in this study are slightly lower than those previously reported for fathead minnows. Holcombe et al. [11] reported a flow-through 96-h LC50 of 5.4 µg/L for technical-grade fenvalerate (no solvent carriers). Other values, derived from static exposures with solvent carriers, are also somewhat higher. Holcombe et al. [11] cite unpublished Columbia National Fishery Laboratory static 96-h LC50s of 2.15 to 2.35 µg/L. Flow-

through 96-h LC50s for technical-grade fenvalerate of 5.0, 0.58 and 0.31 μg/L have been reported for sheepshead minnow (*Cyprinodon variegatus*), striped mullet (*Mugil cephalus*) and Atlantic silversides (*Menidia menidia*), respectively [15]. Holcombe et al. [11] reported a flow-through 96-h LC50 for the rainbow trout of 2.1 μg/L. Flow-through tests with permethrin and flucythrinate (AC222,705; structurally very similar to fenvalerate) yielded 96-h LC50 values for fathead minnows of 15.6 [11] and 0.22 μg/L [16], respectively. Coats and O'Donnell-Jeffery [2] reported a static fenvalerate 24-h LC50 of 76 μg/L (nominal) for rainbow trout, whereas McLeese et al. [17] reported a static 96-h LC50 of 1.2 μg/L for Atlantic salmon. In static tests with EC formulations of the insecticide, using rainbow trout, a 24-h LC50 of 21 μg/L (nominal) [2] and a 48-h LC50 of 3 μg/L [18] have been reported.

The concentration of fenvalerate residues in fathead minnows in this study increased as the exposure concentrations were increased to elicit significant mortality. Residue levels associated with mortality decreased slightly with higher water concentrations. The pattern of accumulation observed in this study is similar to that reported by McLeese et al. [17] for Atlantic salmon. The observed pattern of residue accumulation is consistent with generally accepted views regarding the distribution of lipophilic insecticides (a log p for fenvalerate of 6.2 has been reported [15]) in vertebrates [19] and is compatible with results from studies of the uptake and distribution of [^{3}H]fenvalerate [unpublished data, Bradbury et al.] and [^{14}C]permethrin [20] in rainbow trout. Lethal fenvalerate exposure levels resulted in concentrations in the nervous system that resulted in toxic symptoms and death. This critical level for fathead minnows corresponded to about 1,000 ng/g fenvalerate on a whole-body wet-weight basis. At increasingly higher lethal exposures, the initial insult to the nervous system was greater, thereby resulting in a shorter time to mortality and slightly lower residues.

CFs in the fish from this study were greater for lower water concentrations (and concomitant longer exposure times). This pattern is similar to the CF data reported by McLeese et al. [17]. Although not a classic measure of bioaccumulation, the mean CF derived for the fish that survived dosing, 1,670 ($n = 3$ exposure levels), provides a rough estimate of a fenvalerate bioconcentration factor in fathead minnows. This CF compares with a bioconcentration factor of 3,200 reported for fathead minnows at the end of an early life stage study [21]. Ohkawa et al. [22] reported a value of about 1,000 for carp after a 7-d exposure to the (*S*)-acid isomers of the insecticide, whereas Hansen et al. [23] reported a mean fenvalerate bioconcentration factor of 570 for sheepshead minnows Bioconcentration factors for permethrin and flucythrinate in fathead minnows of 2,800 and 4,000 [15], respectively, are also similar to the upper limit CF values presented here.

The primary objective of this study was to evaluate the influence of emulsifiers on the toxicity and uptake of fenvalerate by fish. The data generated indicate that under flow-through conditions emulsifiers reduce the uptake rate of the insecticide. The reduced uptake seemingly resulted in the initially lower toxicity of the EC formulation. The lower rate of uptake may have been due to an interaction of the insecticide with the emulsifying agents, perhaps resulting in the formation of a microemulsion, thereby reducing the capacity of the insecticide to be absorbed across the gills, i.e., reduced bioavailability. Alternatively, the emulsifiers may interact directly with gill membranes and reduce fenvalerate uptake. Studies with barbiturates, however, have indicated that if a surfactant is capable of disrupting normal gill membrane structure and function, increased uptake results [24,25].

Results of previous studies using static exposure regimes suggested that commercial emulsifiers enhanced the lethality of pyrethroids to fish [2,3]. The opposite effect noted in our studies may be due to different exposure techniques and procedures. In the static tests, emulsifiers probably enhanced availability of the pyrethroids by diminishing their propensity to adsorb to glass [26] and the fish. Because of a reduction in availability with static tests the nominal and/or initial concentrations of technical pyrethroids required to cause mortality would probably be higher than those required with an emulsifier present. In this study, the diluter system was allowed to equilibrate for several days before fish were introduced, to saturate the glass surfaces and thereby eliminate the confounding factor of the static tests. Increased toxicity of ECs noted in the static tests could have been due to additive toxicity or synergism. Results from the current study, however, indicate that the commercial solvents and emulsifiers found in the fenvalerate EC formulation are not toxic to fathead minnows at concentrations well in excess of those used in both the static and flow-through tests, and, in addition, no synergistic effect was noted.

In contrast to previous findings, the initial lethality of technical-grade fenvalerate was greater than that of a 30% a.i. EC formulation; however, the incipient lethalities of the formulations were similar. This initial difference in toxicity seems to be due to a lower rate of fenvalerate uptake in the presence of emulsifiers and suggests a reduction in the bioavailability of the highly lipophilic insecticide in a flow-through system. Although carrier solvents in toxicity evaluations should be discouraged, these results suggest that their use in well-monitored flow-through tests may have little effect on incipient LC50s but will have an effect on the rate at which the toxic response is manifested. The findings of this study further emphasize the need to investigate the physicochemical interactions from combinations of aquatic pollutants and the resultant impact on biological systems.

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