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Abstract

An in vivo rainbow trout (*Salmo gairdneri*) preparation was used to evaluate the gill uptake and toxicokinetics of [^{3}H]fenvalerate ([R,S]-α-cyano-3-phenoxybenzyl [R,S]-2-[4-chloro-phenyl]-3-methylbutyrate), a synthetic pyrethroid insecticide. Fish were exposed to technical-grade fenvalerate (0.28 or 23 ng/L) or an emulsifiable-concentrate formulation (16 ng/L) for 36 to 48 h. No significant effects of emulsifiers or fenvalerate concentration on uptake were observed. The overall mean gill uptake efficiency was determined to be 28.6 + 4.4%. Following 8- to 48-h depuration periods, carcass and bile contained 80 to 90% and 10 to 20% of the gill-absorbed doses, respectively. Urine, feces and blood each contained less than 2% of the dose. Significant excretion and blood transport of fenvalerate equivalents were completed within 8 to 12 h after termination of exposure. Specific tissues from trout exposed to 0.28 ng/L fenvalerate were analyzed for fenvalerate equivalents. After a 48-h depuration period, bile contained the highest concentration of fenvalerate equivalents (7,000 pg/g), followed by fat (200 pg/g). Remaining tissues contained 15 to 45 pg/g. Analysis of biliary metabolites indicated that the glucuronide of 4′-HO-fenvalerate was the only significant degradation product. Results from the present study suggest that efficient gill uptake does not explain the extreme sensitivity of fish to fenvalerate. Rather, a low rate of biotransformation and excretion may play a significant role in the susceptibility of rainbow trout to the synthetic pyrethroid insecticides.

Keywords

Fenvalerate, Salmo gairdneri, Gill uptake, Toxicokinetics, Metabolism

Disciplines

Aquaculture and Fisheries | Entomology | Natural Resources Management and Policy | Toxicology

Comments

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TOXICOKINETICS OF FENVALERATE IN RAINBOW TROUT (*SALMO GAIRDNERI*)

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Abstract—An in vivo rainbow trout (*Salmo gairdneri*) preparation was used to evaluate the gill uptake and toxicokinetics of [^{3}H]fenvalerate ([R,S]-α-cyano-3-phenoxybenzyl [R,S]-2-[4-chlorophenyl]-3-methylbutyrate), a synthetic pyrethroid insecticide. Fish were exposed to technical-grade fenvalerate (0.28 or 23 ng/L) or an emulsifiable-concentrate formulation (16 ng/L) for 36 to 48 h. No significant effects of emulsifiers or fenvalerate concentration on uptake were observed. The overall mean gill uptake efficiency was determined to be 28.6 ± 4.4%. Following 8- to 48-h depuration periods, carcass and bile contained 80 to 90% and 10 to 20% of the gill-absorbed doses, respectively. Urine, feces and blood each contained less than 2% of the dose. Significant excretion and blood transport of fenvalerate equivalents were completed within 8 to 12 h after termination of exposure. Specific tissues from trout exposed to 0.28 ng/L fenvalerate were analyzed for fenvalerate equivalents. After a 48-h depuration period, bile contained the highest concentration of fenvalerate equivalents (7,000 pg/g), followed by fat (200 pg/g). Remaining tissues contained 15 to 45 pg/g. Analysis of biliary metabolites indicated that the glucuronide of 4′-HO-fenvalerate was the only significant degradation product. Results from the present study suggest that efficient gill uptake does not explain the extreme sensitivity of fish to fenvalerate. Rather, a low rate of biotransformation and excretion may play a significant role in the susceptibility of rainbow trout to the synthetic pyrethroid insecticides.

Keywords—Fenvalerate *Salmo gairdneri* Gill uptake Toxicokinetics Metabolism

INTRODUCTION

Pyrethroid insecticides, including fenvalerate ([R,S]-α-cyano-3-phenoxybenzyl [R,S]-2-[4-chlorophenyl]-3-methylbutyrate), are very toxic to fish. Fenvalerate flow-through 96-h LC50 values for rainbow trout (*Salmo gairdneri*) and fathead minnows (*Pimephales promelas*) of 0.5 to 2 μg/L have been reported [1,2]. Possible explanations for the high toxicity of pyrethroids to fish include (a) sensitivity at the site(s) of action, (b) highly efficient gill uptake and (c) inefficient metabolism and elimination.

Efficient uptake of insecticides across the gills and into the bloodstream can result in high toxicity to fish. Water solubility and lipophilicity, parameters generally accepted to influence uptake, have been correlated with the toxicity of insecticides [3], including pyrethroids [4]. Emulsifiers have been found to influence the lethality of pyrethroids [1,5,6], seemingly through effects on uptake [1]. Because of their lipophilicity (octanol/water partition coefficients [log P] of 6.2 to 7.5) pyrethroids may be efficiently absorbed across the gills [7]; however, specific research supporting this view is not available in the literature.

Insufficient rates of pyrethroid detoxification could also contribute to the lethality of pyrethroids; however, few studies have been published regarding the metabolism of these insecticides in fish. In a qualitative sense, in vitro metabolism of permethrin (3-phenoxybenzyl [R,S] *cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) in fish is similar to that observed in mammalian species [8]. In contrast, other in vitro [9] and in vivo [10] studies of permethrin metabolism in rainbow trout indicate an overall lower

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rate of hydrolysis and oxidation than that noted in mammals and birds, species less sensitive to pyrethroids [11]. Greater fish toxicity for the pyrethroids containing an α-cyano substituent in the benzyl alcohol moiety has been proposed to be the result of less efficient metabolism because lethality trends could not be explained adequately by differences in lipophilicity and uptake [5,6,12]. The absence of toxicokinetic studies with α-cyano pyrethroids in fish makes evaluation of this hypothesis impossible. The research presented here is part of a project designed to investigate the gill uptake and toxicokinetics of pyrethroid insecticides in fish. Fenvalerate was selected as the model compound, and the rainbow trout was used as the test species.

MATERIALS AND METHODS

Two studies were undertaken. The first involved the estimation of the in vivo gill uptake, distribution and elimination of fenvalerate; the second included an examination of the in vivo metabolism of fenvalerate. The respirometer-metabolism chambers, exposure system and surgical procedures used to study the uptake and metabolism of fenvalerate were basically as described previously [13–15].

Toxicant preparation and exposure

[^{3}H]Fenvalerate (aromatic) was used in the study (Fig. 1). Technical-grade insecticide, obtained from the Shell Development Company (Modesto, CA), was tritiated via a catalytic exchange reaction (Amersham Corp., Arlington Heights, IL) and isolated from the crude reaction mixture by preparative thin-layer chromatography (TLC) and radioautography. The [^{3}H]fenvalerate had a final radiochemical purity of greater than 98% as determined by TLC, radioautography, liquid scintillation counting (LSC) and gas-liquid chromatography (GLC). The specific activity of the preparation was 5.0 Ci/mmol, as determined by LSC and GLC (see ref. 1 for GLC conditions; TLC systems are described below, under "Analysis of biliary metabolites"). [^{3}H]Fenvalerate with specific activities of 5.0 and 0.11 Ci/mmol (original material diluted with unlabeled insecticide) was used in the metabolism and uptake experiments, respectively. The material was dissolved in benzene:toluene (99:1, v/v) and stored at 4°C until used.

A fresh stock solution of [^{3}H]fenvalerate was prepared with distilled water in an 18-liter stock bottle for each exposure period. Required aliquots of the insecticide were added to the stock bottle, followed by evaporation of the solvent to near dryness. After addition of distilled water, the contents were agitated with a Teflon-coated magnetic stir bar. To test the effect of emulsifiers on the uptake of fenvalerate, a 30% active ingredient (a.i.) emulsifiable concentrate (EC) was formulated by adding the placebo EC (commercial EC with less active ingredient, supplied by the Shell Development Company) at the proper nominal level to [^{3}H]fenvalerate-water solutions.

[^{3}H]Fenvalerate stock solutions were delivered to the toxicant-mixing cell at a rate of 2 ml/min with an FMI (Fluid Metering, Inc., Oyster Bay, NY) chemical-metering pump. The stock solutions were then diluted with Lake Superior water flowing at a rate of 600 or 1,000 ml/min. The fenvalerate solutions flowed into two replicate metabolism chambers at a rate of 300 or 500 ml/min. Mean fenvalerate aqueous concentrations (±SE) were maintained at 23.2 ± 5.1, 15.6 ± 1.6 and 0.28 ± 0.09 ng/L in the technical-uptake, EC-uptake, and metabolism exposures, respectively.

Unfiltered Lake Superior water was maintained at 11.0 to 11.5°C. Overall means and standard deviations ($n = 8$) for hardness and alkalinity [16] were 42.59 ± 0.62 and 44.62 ± 1.20 mg/L as $CaCO_3$, respectively. Dissolved oxygen (DO), measured with a Beckman oxygen electrode (Beckman Instruments, Inc., Arlington Heights, IL), ranged from 10.5 to 11.0 ($n = 224$). Mean pH ($n = 8$) was 7.79 ± 0.06.

Fish preparation

A total of 12 trout were used in the project; each fish was treated as an experimental unit. Four

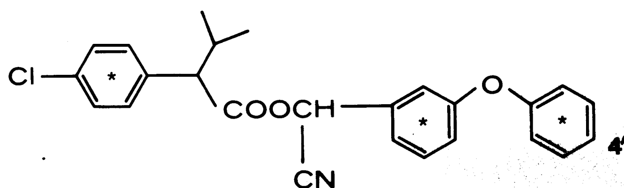

Fig. 1. Structure of fenvalerate, showing ^{3}H-labeling positions (*) and site (4') of metabolic oxidation in rainbow trout.

rainbow trout were used in the metabolism study (two female and two male), whereas in the uptake study, four trout were used with each formulation (technical-grade, three female and one male; EC, one female and three male). The trout were maintained at the U.S. Environmental Protection Agency Environmental Research Laboratory–Duluth (Duluth, MN) for several months before use and acclimated to a temperature of 11 to 12°C. The fish weighed between 0.640 and 0.971 kg and were kept on a 12-h photoperiod (incandescent lighting, 11.0 lm at water surface) during an experiment. Trout food (Glencoe Mills, Glencoe, MN) was withheld from the fish 24 h before use, and fish were not fed during an experiment.

Fish were exposed to fenvalerate in Plexiglas respirometer-metabolism chambers [15] and surgically prepared as previously reported [13,14].

Sampling

In the metabolism study, fish were exposed to the insecticide for 46 to 47 h. Following exposure and flushing of the chambers with untreated water (1 h), depuration of the insecticide was monitored for an additional 48 h. In the uptake study, exposure periods of 36 to 48 h were utilized. Elimination was monitored for 48 h in the technical test. Based on the distribution and elimination data for this group, the fish in the EC test were killed 8 h after exposure. During each exposure period, eight measurements of ventilation rate, ventilation volume, DO uptake (see ref. 15 for methods), and fenvalerate uptake were made for each fish. Gill oxygen- and fenvalerate-uptake efficiency is the percentage decrease in concentration between the inspired and expired water. During depuration phases of an experiment, these parameters (fenvalerate gill elimination rather than uptake) were measured eight times, except in the EC-uptake experiment, for which three measurements were made. Control DO and respiratory function measurements were made prior to exposure to toxicant ($n = 4$ measurements per fish).

Urine (2 ml minimum sample) was collected from each fish at various times throughout the exposure and depuration periods. At the end of the depuration period, the fish were killed by anesthetic overdose, and fecal material was collected via catheter. Blood (5 to 6 ml) was collected by caudal puncture; subsamples were taken to determine total blood radioactivity. The blood was then separated into plasma and packed-cell fractions by centrifugation. Bile (1.0 to 1.5 ml) was collected by gall bladder puncture. In the metabolism study, the entire brain, heart, kidney, liver, ovaries, spleen and testes [1] were removed and weighed. Samples of fat (0.1 to 0.7 g), gill (1.5 to 2.5 g) and muscle (3.5 to 5.5 g) also were removed and weighed. The mass of the remaining carcass was then determined and homogenized. In the uptake experiments, the carcass was homogenized after removal of blood and bile. All tissue samples were stored at −20°C until analyzed.

Water and tissue analysis

[^{3}H]Fenvalerate was measured in inspired and expired water. A 225-ml volume of water was collected in a 250-ml volumetric flask containing 25 ml hexane. After 45 min of vigorous stirring, 10 ml hexane was collected in a scintillation vial and concentrated to 2 to 3 ml. Fifteen milliters of NA cocktail (Beckman) was added before LSC analysis. Extraction of water samples spiked at levels comparable to those in the actual tests resulted in $105 \pm 2\%$ ($n = 8$) recovery. During depuration periods in the metabolism study, expired water was reextracted with ethyl acetate in an attempt to recover any radioactivity associated with more polar metabolites. Ten milliters of MP cocktail (Beckman) was used to count aliquots of the ethyl acetate extract.

[^{3}H]Fenvalerate equivalents were determined in three to four subsamples from each tissue matrix collected from the fish. Urine samples (1 ml) were counted directly in 10 ml MP cocktail. The remaining matrices were analyzed following homogenization and solubilization. Whole blood (250 μl), packed cells (100 μl), plasma (100 μl), bile (25 μl), carcass (150 to 250 mg) and remaining tissues (150 to 250 mg) were digested in glass scintillation vials (3 to 18 h at 40 to 50°C) with 1 to 2 ml of a 1:1 (v/v) mixture of BTS-450® tissue solubilizer (Beckman) and isopropanol. After digestion, 30% H_2O_2 (500 μl) was added with further heating (1 to 3 h) to decolorize the samples. Digests were counted after the addition of 100 to 200 μl glacial acetic acid and 15 ml MP cocktail. Fecal samples were mixed by hand, and samples of the suspension (100 to 125 mg) were then oxidized in LSC vials with 200 μl 60% perchloric acid and 400 μl 30% H_2O_2 at 60°C for 3 h. After cooling, 15 ml MP cocktail was added. Spiked samples indicated no loss of tritium after heating and resulted in $108 \pm 4\%$ ($n = 8$) recovery.

Radioactivity in water and tissue samples was quantified using either a Beckman LC-8000 series or an LKB 1217 liquid scintillation counter (LKB Instruments, Inc., Rockville, MD). Both counters

were equipped with computerized dpm packages. Quench correction was based on external standard ratio (LKB) or H number (Beckman) techniques. Water and tissue samples were counted to 1% and 1 to 7% uncertainty, respectively.

Analysis of biliary metabolites

Bile collected from each fish used in the metabolism study (1.5 to 2.0 ml per fish) was analyzed individually by methods adapted from Glickman et al. [10]. Following acidification to a pH of 1.5 with HCl, the bile samples were extracted three times with an equal volume of ethyl acetate. The extract was then dried over Na_2SO_4. Between 80 and 87% of the radioactivity was extracted from the bile. Aliquots of the bile extract were then exposed to β-glucuronidase (Sigma Chemical Co., St. Louis, MO) in appropriate buffer systems with proper controls (including saccharic acid 1,4-lactone; see ref. 17 for methods). Additional aliquots were incubated with aryl sulfatase (Sigma). After incubation and acidification with HCl, the enzyme–bile extract solutions were extracted three times with equal volumes of ethyl ether: 95% ethanol (3:1, v/v) and dried over Na_2SO_4. The concentrated extracts were then applied to TLC plates after addition of unlabeled standards.

TLC utilized silica gel 60 F-254 20 × 20 cm plates with a thickness of 0.25 mm (MCB Reagents, Gibbstown, NJ) and the following two solvent systems (by volume): A, hexane:toluene:acetic acid (3:15:1), two developments; B, benzene (saturated with formic acid):ethyl ether (10:3), two developments. All solvents were reagent-grade. The following unlabeled standards were used: *m*-phenoxybenzyl alcohol, *m*-phenoxybenzaldehyde and *m*-phenoxybenzoic acid (all purchased from Aldrich Chemical Co., Milwaukee, WI); 2-(*p*-chlorophenyl)-3-methylbutyric acid (purchased from Frinton Laboratories, Vineland, NJ); fenvalerate, 4′-HO-fenvalerate and 4′-HO-*m*-phenoxybenzoic acid (all generously provided by the Shell Development Company). Parent compound and the metabolites were separated using two-dimensional chromatography (system A followed by system B; method adapted from ref. 18). Standards were visualized with ultraviolet light (254 nm). Radioactive sites on the plates were detected by radioautography (LKB Ultrofilm ^3H; LKB Instruments) and confirmed and quantified by scraping appropriate segments of the plates and measuring by LSC. Biliary metabolites were identified through cochromatography with unlabeled standards.

Statistical analysis

To compare respiratory function, fenvalerate uptake and fenvalerate distribution data, *t* tests were used. Regression analysis was used to evaluate uptake efficiency over time [19]. A *p* value of 0.05 was used to determine significance.

RESULTS

Respiratory function data obtained in each study are listed in Table 1. Within each test, there was no significant difference between preexposure, exposure and depuration values, suggesting no sublethal effect of the insecticide on respiratory function. There also was no difference in respiratory parameters between fish in the technical- and EC-uptake studies. The respiratory parameters from the 12 fish are similar to values obtained previously for transected rainbow trout [13,14], but the overall mean ventilation volume was 1.5 times higher.

Uptake study

Mean gill fenvalerate-uptake efficiencies for the fish in the technical and EC groups were 23.6 and 30.3%, respectively (Table 2). No significant difference between uptake efficiencies for the two formulations was noted. One fish in the EC group had a substantially higher ventilation volume (22.2 L/h) and fenvalerate-uptake efficiency (39.2%) than did the remaining three fish. EC-uptake data, excluding this aberrant fish, are also included in Table 2. Regression analysis of fenvalerate uptake over time indicated that within each formulation uptake efficiency did not vary (slopes were not significantly different from 0). During the depuration periods, no measurable elimination of fenvalerate across the gills was noted.

The total [^3H]fenvalerate equivalents absorbed by each fish were calculated by multiplying the aqueous fenvalerate concentration by gill uptake efficiency, ventilation volume and total exposure time. Dividing this value by fish weight gave a calculated concentration in an individual, assuming no excretion. Dividing this concentration by exposure time provided a calculated dose rate of [^3H]fenvalerate on a ng/kg/h basis. Mean values for each formulation are listed in Table 2. With exclusion of the aberrant fish, a mean dose rate of 48.1 ng/kg/h was determined for the EC group, whereas a dose rate of 74.9 ng/kg/h was calculated for the technical group. The lower dose rate in the EC group is a function of the proportionately lower fenvalerate exposure concentration.

By quantifying the [^3H]fenvalerate equivalents

Table 1. Comparison of respiratory function in rainbow trout before, during and after aqueous exposure to [^{3}H]fenvalerate[a]

	Preexposure	Exposure	Depuration
Ventilation rate (no./min)			
Uptake study			
Technical formulation[b]	58 ± 10	59 ± 13	60 ± 15
EC formulation[c]	72 ± 4	70 ± 2	69 ± 5
Metabolism study[d]	60 ± 5	63 ± 7	60 ± 6
Overall mean	64 ± 9	63 ± 9	63 ± 10
Ventilation volume (L/h)			
Uptake Study			
Technical formulation	9.24 ± 1.98	11.10 ± 2.40	11.52 ± 2.10
EC formulation	13.74 ± 6.12	13.70 ± 6.60	14.80 ± 6.60
Metabolism study	8.76 ± 1.32	8.73 ± 2.22	8.82 ± 2.10
Overall mean (L/h)	10.92 ± 4.08	11.12 ± 4.26	11.76 ± 4.56
Overall mean (L/kg/h)	12.47 ± 6.88	13.76 ± 6.57	13.84 ± 6.50
Oxygen uptake efficiency (%)[e]			
Uptake study			
Technical formulation	57 ± 16	53 ± 18	49 ± 15
EC formulation	48 ± 20	47 ± 20	45 ± 20
Metabolism study	62 ± 9	60 ± 13	61 ± 12
Overall mean	58 ± 15	54 ± 16	52 ± 16

[a]Mean ± standard error for $n = 4$ fish per experiment ($n = 12$ for overall means). Functions were measured four, eight and three to eight times per fish during preexposure, exposure and depuration periods, respectively.
[b]Fish were exposed to 23.2 ± 5.1 ng/L fenvalerate.
[c]Fish were exposed to 15.6 ± 1.6 ng/L fenvalerate.
[d]Fish were exposed to 0.28 ± 0.09 ng/L fenvalerate.
[e]O_2 uptake efficiency = [(inspired [O_2] − expired [O_2])/(inspired [O_2])] × 100%.

in each fish and dividing by fish weight and exposure time, measured dose rates (Table 3) were determined to evaluate the accuracy of the calculated dose rates. Mean measured dose rates of 61.7 and 41.6 ng/kg/h were determined for the technical and EC groups, respectively. The distribution of [^{3}H]fenvalerate equivalents based on the calculated dose is presented in Table 4, and no significant difference was noted between formulations. Overall, the mean measured doses were about 82% that of the calculated doses. Based on the calculated dose, the highest percentage of [^{3}H]fenvalerate equivalents was associated with the remaining carcass (about 73%), followed by bile (about 7.5%). Blood, urine and feces contained between 0 and 1.2% of the dose. Distribution of [^{3}H]fenvalerate equivalents based on the measured dose was also determined (data not shown). In both groups, about 88% of the measured dose was in the remaining carcass, whereas the bile contained about 10% of the dose. Blood, feces and urine contained between 0 and 1.5% of the measured dose.

With both formulations, elimination and blood transport of [^{3}H]fenvalerate equivalents were nearly completed within 8 to 12 h after termination of exposure. In the technical-uptake test, whole-blood radioactivity was not detected (0.38 ng/ml) after a 48-h depuration period, whereas in the EC-uptake study, 0.5% of the dose was measured in the whole blood after 8 h of depuration. Of this amount, 88 ± 5% was associated with the packed cells and 12 ± 6% was associated with the plasma. In both studies, a similar urine excretion pattern was noted. Radioactivity was not detectable in urine samples until 12 to 20 h after initiation of exposure. The concentration of [^{3}H]fenvalerate equivalents then increased with time. Peak concentrations were reached during the final 1 to 2 h of exposure or during the first 1 to 2 h of the depuration period, after which concentrations steadily decreased. Levels were still detectable after 8 h of depuration in the EC-uptake study; in the technical group (48-h depuration period), urine radioactivity was no longer detectable (0.1 ng/ml) between 12 and 30 h into the depuration period.

Metabolism study

A mean gill fenvalerate-uptake efficiency of 32.1% (Table 2) in this study was not significantly

Table 2. Calculated [^{3}H]fenvalerate dose received by rainbow trout during aqueous exposure[a]

[^{3}H]Fenvalerate equivalents in water (ng/L)	[^{3}H]Fenvalerate uptake efficiency[b]	Ventilation volume (L/h)	Exposure time (h)	Total [^{3}H]fenvalerate equivalents[c] (ng)	Fish weight (kg)	[^{3}H]Fenvalerate equivalents concentration in whole fish (ng/kg)	Dose[d] (ng/kg/h)
Uptake study							
Technical formulation							
23.2 ± 5.1	0.236 ± 0.10	1.11 ± 2.4	47.8 ± 0.3	2,740 ± 910	0.777 ± 0.124	3,580 ± 1,190	74.9 ± 24.9
Emulsifiable concentrate formulation[e]							
15.6 ± 1.6	0.303 ± 0.064	13.7 ± 6.6	41.8 ± 6.6	2,690 ± 1,660	0.860 ± 0.098	3,290 ± 2,430	78.9 ± 58.2
(15.3 ± 1.9)	(0.273 ± 0.029)	(10.8 ± 4.0)	(43.7 ± 6.6)	(1,860 ± 196)	(0.897 ± 0.078)	(2,090 ± 348)	(48.1 ± 8.8)
Metabolism study							
0.28 ± 0.09	0.321 ± 0.132	8.73 ± 1.31	46.5 ± 0.3	35.6 ± 13.9	0.894 ± 0.045	39.4 ± 17.1	0.85 ± 0.37

[a]Mean ± standard error based on $n = 4$ fish per experiment. Water concentrations, uptake efficiencies and ventilation volumes were determined eight times per fish during an exposure period.
[b]Uptake efficiency = (inspired [^{3}H]fenvalerate − expired [^{3}H]fenvalerate)/inspired [^{3}H]fenvalerate.
[c]Water concentration × uptake efficiency × ventilation volume × exposure time, calculated for each fish separately.
[d][^{3}H]Fenvalerate equivalent concentration/exposure time.
[e]Ventilation volume and fenvalerate uptake for one fish in this experiment were substantially higher than those noted in the other fish. The data in parentheses exclude the aberrant fish, i.e., $n = 3$.

Table 3. Measured [^{3}H]fenvalerate dose received by rainbow trout during aqueous exposure[a]

[^{3}H]Fenvalerate Equivalents in remaining carcass[b] (ng)	[^{3}H]Fenvalerate equivalents in whole blood (ng)	[^{3}H]Fenvalerate equivalents in bile (ng)	[^{3}H]Fenvalerate equivalents in feces (ng)	[^{3}H]Fenvalerate equivalents in urine (ng)	Total [^{3}H]fenvalerate equivalents (ng)	[^{3}H]lFenvalerate equivalents concentration in whole fish (ng/kg)	Dose rate[c] (ng/kg/h)
Uptake study							
Technical formulation (23.2 ng/L)							
1,994 ± 599	ND[d]	213 ± 132	16.5 ± 11.3	24.5 ± 18.7	2,250 ± 630	2,950 ± 878	61.7 ± 18.1
Emulsifiable concentrate formulation (15.6 ng/L)							
1,260 ± 244	7.55 ± 0.47	142 ± 88	7.20 ± 0.90	20.9 ± 2.9	1,437 ± 236	1,682 ± 310	41.6 ± 13.1
Metabolism study (0.28 ng/L)							
20.43 ± 2.96	0.12 ± 0.04	6.62 ± 1.40	0.10 ± 0.12	0.98 ± 0.51	27.9 ± 4.1	31.2 ± 4.9	0.67 ± 0.10

[a]Mean ± standard error based on $n = 4$ fish per experiment.
[b]Remaining carcass includes various organs and tissues removed (see text). Levels determined after 48-h (technical-uptake and metabolism study) or 8-h (EC-uptake study) depuration, except for urine, which was monitored throughout the exposure and depuration periods.
[c]Dose rate = total [^{3}H]fenvalerate equivalents/fish weight/exposure period.
[d]Not detectable. Limit of detection for blood in this study was 0.38 ng/ml.

Table 4. Distribution of [^{3}H]fenvalerate equivalents in rainbow trout as percentage of calculated dose[a]

	Uptake study		
Tissue	Technical formulation[b] (23.2 ng/L)	EC formulation[c] (15.6 ng/L)	Metabolism study[b] (0.28 ng/L)
Remaining carcass	74.1 ± 7.4	72.3 ± 2.5	66.6 ± 28.4
Blood	ND[d]	0.4 ± 0.04	0.4 ± 0.3
Bile	7.8 ± 4.6	7.5 ± 6.0	21.3 ± 8.4
Feces	0.7 ± 0.6	0.4 ± 0.1	0.4 ± 0.5
Urine	0.9 ± 0.8	1.2 ± 0.3	2.3 ± 0.5
Total	85.3 ± 5.0	81.8 ± 4.8	91.0 ± 36.1

[a]See Table 2 for calculated doses.
[b]Mean ± standard deviation based on $n = 4$ fish.
[c]Mean ± standard deviation based on $n = 3$ fish. One fish had an abnormally high ventilation volume and fenvalerate uptake efficiency (see Table 2 and text), and data from this fish were excluded. Total recovery of the calculated dose for this fish was 22.6%.
[d]Not detectable. Limit of detection for blood in this study was 0.38 ng/ml.

different from that determined in the uptake study. The mean calculated dose rate for the four fish was 0.85 ng/kg/h (Table 2), and the mean measured dose rate was determined to be 0.67 ng/kg/h (Table 3). Agreement between calculated and measured doses (Table 4) was similar to that in the uptake study; however, the between-fish variability at the lower fenvalerate exposure level was higher.

On a gross level, the distribution of [^{3}H]fenvalerate equivalents in these fish was similar to that described in the uptake study (Table 4). The percentage of fenvalerate equivalents was highest in the remaining carcass, followed by bile, urine, feces and blood. With the lower fenvalerate exposure level in the metabolism study, a significantly greater proportion of the measured dose was associated with the bile (24% versus 10% in the uptake study). Correspondingly, a significantly lower percentage of measured equivalents was noted in the carcass fraction in the metabolism study (70%) than in the uptake study (90%). The percentage of fenvalerate equivalents in the urine fraction and the time course of excretion were similar to those noted in the uptake study. Elimination of [^{3}H]fenvalerate equivalents across the gills was not detected. As observed in the previous study, the packed cell fraction of the blood had a greater percentage of whole-blood radioactivity (packed cells, 78 ± 7%; plasma, 22 ± 8%).

The concentration of [^{3}H]fenvalerate equivalents in a variety of tissues was determined after completion of the depuration period (Table 5). Bile concentrations (about 7,000 pg/g) were substantially higher than those determined in any other matrix. Concentrations in the fat of about 200 pg/g were 50 to 100 times higher than those in remaining tissues. Of the remaining tissues, slightly higher concentrations were found in the gills, liver, kidneys and packed blood cells (about 45 pg/g).

Table 5. Concentration of [^{3}H]fenvalerate equivalents in rainbow trout tissues after a 48-h aqueous exposure (0.28 ng/L) and 48-h depuration

Tissue	[^{3}H]Fenvalerate equivalents (pg/g)[a]
Bile	7,060 ± 4,910
Blood (whole)	22 ± 7
Brain	21 ± 11
Carcass (remaining)	23 ± 4
Fat	203 ± 47
Gill	46 ± 22
Heart	17
Kidney	44 ± 10
Liver	49 ± 7
Muscle	27 ± 4
Ovaries	27
Plasma	8 ± 2
Red blood cells	46 ± 12
Spleen	34 ± 9
Testes	ND[b]

[a]Mean ± standard deviation for $n = 4$ fish, except for heart, ovaries and testes, for which $n = 2$. The range for heart and ovaries was 13–21 and 25–30 pg/g, respectively.
[b]Not detectable. Limit of detection for testes was 10 pg/g.

Analysis of biliary metabolites indicated that the majority of the radioactivity was associated

with a glucuronide conjugate(s), inasmuch as 98% was displaced to a higher R_F following treatment with β-glucuronidase. Of the radioactivity released, 91% cochromatographed with 4′-HO-fenvalerate (Fig. 1). Based on the measured dose and the amount of extractable activity in the bile, a mean of 18% of the absorbed dose was associated with this ester metabolite. Significant quantities of hydrolyzed metabolites were not detected.

DISCUSSION

The mean gill fenvalerate-uptake efficiencies derived from the uptake and metabolism studies were not significantly different, resulting in an overall mean of 28.6 ± 4.4%. The independence of gill absorption from fenvalerate concentration (0.28 to 23 ng/L) is consistent with observations for endrin [15] and a polychlorinated biphenyl isomer [14]. To verify the accuracy of derived uptake efficiencies, calculated doses were compared with doses obtained from measuring and summing fenvalerate levels in various tissue compartments. Measured doses were in reasonable agreement with calculated doses, which further establishes that the total flux of a chemical across the gills is a function of water concentration, uptake efficiency and respiratory volume [14].

The gill uptake efficiency value for fenvalerate is consistent with an in vivo passive-diffusion model of xenobiotic absorption. McKim et al. [13], using rainbow trout, studied the gill uptake of 14 different chemicals as related to log *P* (less than 1 to 7.5). Results for fenvalerate, at a calculated log *P* of 7.2 [20], agree closely with those for mirex (log *P* of 7.5; 20% uptake efficiency) and indicate that, at log *P* values between 6 and 7, gill uptake efficiency drops from about 60% to 20 to 30%.

Results from a previous study indicated that, at lethal aqueous concentrations (0.5 to 10.0 μg/L), fenvalerate uptake in fathead minnows, based on whole-body residues, was approximately twice as rapid with a technical formulation than with a 30% a.i. EC formulation [1]. In the present study, no significant effect of the commercial emulsifier was observed. Aqueous fenvalerate concentrations used in the previous study were approaching water-solubility limits and, at those levels, significant interactions with emulsifying agents may result. In the present study, fenvalerate concentrations were well below solubility limits.

The distribution of [^{3}H]fenvalerate equivalents indicates that fenvalerate and its metabolites are not readily eliminated by rainbow trout. In addition, any significant excretion and transport must occur within hours of exposure termination. These findings are generally similar to those noted for permethrin after aqueous and i.p. exposures in rainbow trout [10], with some indication that fenvalerate and its metabolites may be less readily eliminated. In contrast, warm-blooded vertebrates eliminate fenvalerate and its metabolites very efficiently. Following oral administration over 5 consecutive d, male rats excreted nearly 90% of the dose within 1 to 2 d after exposure [18]. Bobwhite quail, administered fenvalerate orally for 14 d, reached steady-state excretion levels of 80 to 90% of the accumulated dose within the first 3 to 4 d of exposure [21]. Some of the differences noted among species could be due to the route of exposure; presumably, an i.v. exposure in mammals would more closely mimic gill-uptake exposure in fish. No studies of the metabolism of fenvalerate following an i.v. exposure are available; however, the metabolism of deltamethrin ([S]-α-cyano-3-phenoxybenzyl [1R,3R]-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate) in rats after i.v. exposure [22] seems similar to that noted following oral exposure [23].

Analysis of specific tissues for [^{3}H]fenvalerate equivalents after a 48-h depuration period indicates that bile contained the highest concentrations, followed by fat. The high concentration in bile is consistent with its role in excretion. Fenvalerate has been found to concentrate in the fat of mice and rats at levels generally 10 times that noted in other tissues [18,24], which is consistent with results of the present study. Permethrin was also found to concentrate in the fat of rainbow trout [10]. Accumulation in fat is probably a function of the high lipophilicity of fenvalerate and related pyrethroids. The packed-cell fraction of the blood concentrated higher levels of [^{3}H]fenvalerate equivalents than did the plasma. Typically, insecticides are transported in the plasma fraction of the blood [25]. The results of this study further underscore the rapid distribution of fenvalerate into lipid compartments within trout.

Analysis of biliary metabolites indicates that oxidation at the 4′ position and then glucuronidation were the only significant fenvalerate detoxification steps in rainbow trout. Similar detoxification reactions have been reported for cypermethrin ([R,S]α-3-phenoxybenzyl [R,S)*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) [26]. The in vivo metabolism of permethrin in rainbow trout was also qualitatively similar [10]; however, it would seem that the metabolism and

excretion of fenvalerate by rainbow trout are less efficient. This difference in metabolism, probably coupled with differences at the site of action, may contribute to the greater toxicity of fenvalerate to rainbow trout (see refs. 2, 4–6 and 12 for comparative toxicity data). The extent and nature of fenvalerate metabolism in trout are markedly different from those observed in mammals and birds, species moderately to highly insensitive to fenvalerate [27,28]. In rats and mice, 80% of orally administered doses were eliminated in the excrement as various oxidative (including 4′-HO-fenvalerate) and hydrolytic products [18,24]. Similar levels of metabolism have been found in bobwhite quail administered fenvalerate orally [21].

The results of the present study permit an evaluation of potential factors responsible for the sensitivity of rainbow trout to fenvalerate. The evidence indicates that rapid gill uptake does not explain the extreme sensitivity of fish to fenvalerate. Even though gill uptake of fenvalerate is inefficient relative to that of many other xenobiotics, direct uptake of fenvalerate into the bloodstream is an important consideration. The route of administration in mammals does influence lethality; fenvalerate is more toxic to rats after an i.v. exposure (50 to 100 mg/kg; [29]) than after an oral exposure (450 mg/kg [30]). Studies completed with fathead minnows [1] and those in progress with rainbow trout indicate that, after aqueous exposure, whole-body doses of about 0.2 to 1.5 mg/kg fenvalerate are associated with lethality after 12 to 24 h of exposure. If it were assumed that gill-absorbed doses approximate i.v. doses (given the differences in peak blood concentrations), fish still are very sensitive.

Low rates of fenvalerate elimination and metabolism do seem to be contributing factors in the piscicidal activity of fenvalerate, presumably by facilitating greater concentrations of the parent material at the site of action. A reduced rate of permethrin metabolism has been proposed to play a role in the toxicity of permethrin in rainbow trout [9,10] as well. Although metabolism may be involved, the sensitivity of fish at the site of action and their physiological response to intoxication may also be important contributing factors. Fenvalerate, a member of the Type II pyrethroid class, is generally considered to act upon the central nervous system, although the actual site and mode of action are unknown [11]. In a study in which trout ($n = 4$) were exposed to lethal, aqueous concentrations of fenvalerate (about 300 μg/L), 100% mortality occurred in 10.5 ± 1.9 h. Brain fenvalerate residue levels at death were 0.16 ± 0.05 μg/g [1]. These residues correspond to brain fenvalerate concentrations in bobwhite quail (an insensitive species) associated with 20 to 30% mortality about 24 h after oral administration of 500 to 1,000 mg/kg fenvalerate [27]. These data indicate that trout may be more sensitive at the site of action in the nervous system and/or that an additional non-neural site of action exists in fish. It is hoped that research in progress will help elucidate the toxic mode of action of fenvalerate in rainbow trout.

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