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TOXICITY AND TOXICOKINETICS OF FENVALERATE IN FISH

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Toxicity and toxicokinetics of fenvalerate in fish

by

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GENERAL INTRODUCTION

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History

The second generation of synthetic pyrethroid insecticides maintain high insecticidal activity while achieving a significant degree of photostability (1). Elliott et al. (2) attained the initial breakthrough with the development of permethrin (3-phenoxybenzyl [R,S] cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate). These esters, derived from the photostabilized cis and trans dichlorovinyl analogs of chrysanthemic acid and 3-phenoxybenzyl alcohol, have a half-life of several days in the sun, rather than hours as was common with the earlier pyrethroids (2). Development of esters containing an α-cyano-3-phenoxybenzyl alcohol moiety led to the discovery of a number of new compounds, including cypermethrin ([R,S] α-cyano-3-phenoxybenzyl [R,S] cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) and deltamethrin ([S] α-cyano-3-phenoxybenzyl cis-[1R,3R]-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate), which were found to be more potent than the 3-phenoxybenzyl analogs (3,4,5).

Research in Japan also resulted in the discovery of the potent α-cyano analogs of phenoxybenzyl alcohol (6). It was also disclosed that α-isopropylphenylacetates, due to common structural features with dimethylcyclopropanecarboxylates, maintain high levels of insecticidal activity (7,8). This research culminated most notably in the development of fenvalerate, a potent synthetic pyrethroid (6,7). Fenvalerate, marketed

under the name Pydrin® in the United States by the Shell Chemical Company, is currently registered for use on a variety of crops in Iowa and the United States (9).

During the late 1970s and early 1980s additional compounds were described. Many, including flucythrinate ([R,S] α-cyano-3-phenoxybenzyl [R,S] 2-(4-(difluoromethoxy)phenyl)-3-methylbutyrate) and fluvalinate ([R,S] α-cyano-3-phenoxybenzyl [R,S] 2-(2-chloro-4-triofluoromethylphenylamino)-3-methylbutanoate), are noteworthy for their fluorination (10). Oxime and hydroxylamine ether pyrethroids (11), diesters of the nor-pyrethric acid series (12), and biphenylmethyl pyrethroids (13) are examples of recently reported advances in pyrethroid chemistry.

Species sensitivity

While pyrethroids are up to four to five times more toxic to many insects than carbamate, organochlorine and organophosphate insecticides (14), they are generally of low to moderate toxicity to mammals and birds (15). The acute LD_{50}s of technical permethrin to rats and mice range from 650 to 430 mg/kg (16). Deltamethrin is more toxic, however, with LD_{50}s to rats of 52 to 31 mg/kg (17). The toxicity of technical fenvalerate to mammals is similar to that of permethrin with acute oral LD_{50}s to rats and mice between 450 and 100 mg/kg (18,19). Birds are significantly less sensitive to pyrethroid poisoning than mammals. The LD_{50}s of permethrin to mallard ducks (Anas platyrhynchos) and Japanese quail (Coturnix coturnix) are in excess of 4,640 (20) and 13,500 mg/kg (21), respectively. Deltamethrin has been reported to be of low acute toxicity to ducks with an

LD_{50} greater than 4000 mg/kg (21). Bradbury and Coats (22) reported low toxicity of fenvalerate to bobwhite quail (Colinus virginianus) as well (LD_{50} > 4000 mg/kg; 8-d dietary LC_{50} > 15,000 ppm).

Although the acute oral toxicity of pyrethroids to terrestrial vertebrates is low to moderate, their toxicity to fish, during aqueous exposure, is quite high. Flow-through 96-h LC_{50} values (measured toxicant concentrations) for fenvalerate, permethrin, and flucythrinate of 0.22 to 5.4 µg/L have been reported for the Atlantic silversides (Mendidia menidia), fathead minnow (Pimephales promelas), rainbow trout (Salmo gairdneri), sheepshead minnow (Cyprinodon variegatus), and striped mullet (Mugil cephalus) (23,24,25). Some studies have indicated that emulsifiers may enhance the toxicity of pyrethroids to fish. Based on static 24-h LC_{50} values (nominal concentrations), emulsifiable concentrate (EC) formulations of permethrin, fenvalerate, cypermethrin, and fenpropathrin ([R,S] α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate) were 2.2, 3.6, 5.0 and 8.9 times, respectively, more toxic to rainbow trout than were the corresponding technical materials (26). Similar results have been obtained with deltamethrin (27). These findings have led some workers to conclude that the causative agent(s) responsible for the enhanced lethality resides in the emulsifiers, even though there was no supporting data, and have suggested the need to reformulate the commercial products (28). Although the studies of Coats and O'Donnell-Jeffery (26) and Zitko et al. (27) imply increased lethality of EC products, several aspects of the studies may have biased the results (see INTRODUCTION of CHAPTER I). Additional research in this area is needed to resolve the matter.

Vertebrate metabolism

To more fully evaluate the toxicology of pyrethroids in vertebrates, investigators have examined the biotransformation and excretion of many of the more potent compounds. Biodegradation results in more polar and, therefore, excretable products. In vivo mammalian studies of the metabolism of [^{14}C]permethrin (29,30), [^{14}C]cypermethrin (31,32,33), [^{14}C]deltamethrin (34,35) and [^{14}C]fenvalerate (36,37,38), following oral administration, indicate that ester hydrolysis is a very significant degradation step. Oxidation reactions are also important. A major site of hydroxylation is the 4' position in the alcohol moiety. Hydroxylation is also reported at the 2' position with cis-permethrin, fenvalerate and deltamethrin and at the 5 position with deltamethrin. Methyl groups in the acid moiety of some pyrethroids are also oxidized. Conjugation of hydroxylated pyrethroid metabolites with glucuronic acid, sulfate, glycine and other sugars and amino acids have been reported. Following oral administration, mammals generally excrete between 90 to 100% of pyrethroid doses within 3 to 12 d post-dose. The metabolism of [^{14}C]permethrin in chickens (39) and [^{3}H]fenvalerate in bobwhite quail (40) is similar to that noted in mammals; however, avian species are seemingly more efficient in degrading and excreting pyrethroids. Within 1 to 3 d post-dose, 90 to 100% of administered radiolabel is recovered in the excrement. This more rapid elimination is likely a major factor in avian tolerance to pyrethroid intoxication (39,40).

Although numerous studies regarding terrestrial vertebrates are available, few studies have been published which address the metabolism of

pyrethroids in fish. In a qualitative sense, *in vitro* metabolism of permethrin in fish is similar to that observed in mammals (41). In contrast, other *in vitro* (42) and *in vivo* (43) studies of the metabolism of permethrin in rainbow trout indicate a lower overall rate of hydrolysis and oxidation than that noted in mammals and birds. *In vivo* trout studies (43) indicated that the glucuronide of 4'-HO-permethrin was the only significant biliary metabolite; similar detoxification steps have been reported in preliminary findings for cypermethrin (44). Little elimination of permethrin metabolites was observed in rainbow trout and about 70% of the gill-absorbed or intraperitoneal (i.p.) administered doses remained in the fish (43). No toxicokinetic studies of an α-cyano pyrethroid in fish is available in the literature.

Mode of action

Distinct classes of pyrethroid poisoning in both mammals and insects have been reported. Type I pyrethroids, generally including the simple phenoxybenzyl esters, are noted for their effects on the peripheral nervous system. The Type II pyrethroid class is comprised of the α-cyano phenoxybenzylic compounds, and they are considered to act upon the central nervous system (45,46,47,48).

The specific target site of the pyrethroid insecticides is an active area of research, and several hypotheses have been developed regarding the primary mode of action. Narahashi and co-workers (49,50) hypothesize that the pyrethroids interact with sodium channels. Type I pyrethroids are proposed to open sodium channels transiently, cause large depolarizing after-potentials, and evoke repetitive firing but elicit no significant

effect on the neuron resting potential. Type II pyrethroids are reported to open sodium channels persistently, resulting in membrane depolarization, a block of action potentials, and no repetitive firing. Matsumura and Clark (51,52) propose that inhibition of various neural Ca-ATPases is the key step in pyrethroid intoxication. Selective inhibition of Ca-ATPase(s) responsible for regulating calcium permeability across neuron membranes, by Type I pyrethroids, is suggested to lead to membrane destabilization. Inhibition of Ca-Mg-ATPases, ATPases responsible for sequestering intracellular calcium, by Type II pyrethroids is proposed to lead to synaptic facilitation due to increased transmitter release. More recently, Casida and co-workers (53,54,55) have proposed that the toxicity of Type II pyrethroids can be explained by a non-competitive binding to a site in the α-aminobutyric acid (GABA)-receptor-ionophore complex in vertebrate brain synaptosomes. This binding leads to a block in chloride influx, which results in hyperexcitability.

Only one study has been published in which the role of target sensitivity has been addressed with regard to the piscicidal activity of pyrethroids. Glickman and Lech (56), using permethrin as a model compound, reported 3 to 10 fold lower brain residue levels, at death, in rainbow trout than mice. These findings suggest a possible difference in specificity at the site of action in the nervous system. Alternatively, an additional toxic mode of action can be envisioned as a contributing factor in fish sensitivity. Fish utilize a variety ATPases, including Ca-ATPases in the gills (57), to maintain homeostasis in the aqueous environment. It can be postulated, based partially on the work of Clark and Matsumura

(51,52), that the pyrethroid insecticides may significantly interfere with osmoregulatory processes in fish. This physiological insult, in conjunction with nervous system effects, may contribute to the observed sensitivity of fish to the pyrethroid insecticides. McKenny and Hamaker (58) reported that fenvalerate, at lethal and sublethal aqueous concentrations, modified the osmoregulation of an estuarine grass shrimp (Palaemonetes pugio). Leadem et al. (59) and Campbell et al. (60) noted interactions between organochlorine insecticide intoxication in rainbow trout and ion water concentration. At the present time, there is no research available in the literature which specifically addresses the toxic mode of action or physiological response of fish during pyrethroid intoxication.

Development of Objectives

While the acute oral toxicity of the pyrethroids to terrestrial vertebrates is low to moderate, their toxicity to fish during aqueous exposure is high. Possible explanations for the high toxicity of pyrethroids to fish include: 1) sensitivity at the site(s) of action, 2) highly efficient gill uptake, and 3) inefficient metabolism and elimination. Increased piscicidal activity of pyrethroids, when in association with emulsifying agents, has also been reported. Enhanced lethality of pyrethroid EC formulations could result from: 1) additive toxicity, 2) synergism, and 3) enhanced uptake of the active ingredient (possibly due to increased availability). The present study addresses the differential toxicity and uptake of technical and formulated pyrethroids in fish and pyrethroid gill-uptake and toxicokinetics. Fenvalerate was used

as the model compound and the fathead minnow and rainbow trout were used as test species. By further developing a data base involving: 1) contributing factors in the differential toxicity of pyrethroids and 2) their interactions with other chemicals, it is hoped the environmental significance of fenvalerate and the pyrethroids will be better understood.

Explanation of Dissertation Format

This dissertation has been completed following an alternate format, with each chapter comprising a paper submitted to an appropriate scientific journal. Chapter I addresses the differential toxicity of technical and EC formulations of fenvalerate to fathead minnows. This paper has recently been published in Environmental Toxicology and Chemistry (61). The toxicokinetics and gill uptake of fenvalerate in rainbow trout is examined in Chapter II. This paper has been submitted to Environmental Toxicology and Chemistry as well. The Appendix contains data regarding the physiological response of rainbow trout to lethal aqueous concentrations of fenvalerate. Reference sections are included at the end of each chapter. An additional reference section at the end of the dissertation lists sources used in the GENERAL INTRODUCTION and SUMMARY sections. The fish exposure phases of this project were performed at the United States Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, MN in close association with Dr. James M. McKim, a co-author on each paper.

CHAPTER I. 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 LC_{50} 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 LC_{50}s for technical-grade fenvalerate and the EC were 0.69 and 0.99 µg/L, respectively. By 168 h, an LC_{50} 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 LC_{50} 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 (a.i.) (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). 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 x 7.5 x 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 x 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 4710A gas chromatograph equipped with a ^{63}Ni electron-capture detector and a coiled glass column (1.0 m x 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 Environmental 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 LC_{50} 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 LC_{50} values for both formulations initially decreased over time. An LC_{50} 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 LC_{50} values obtained from the EC test continued to decline after 96 h (96-h LC_{50} 0.99 µg/L) and reached an apparent steady state between 120 and 168 h, with a 168-h LC_{50} 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 LC_{50} 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 LC_{50} values were comparable with the technical-grade fenvalerate 96-h LC_{50} (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, 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;

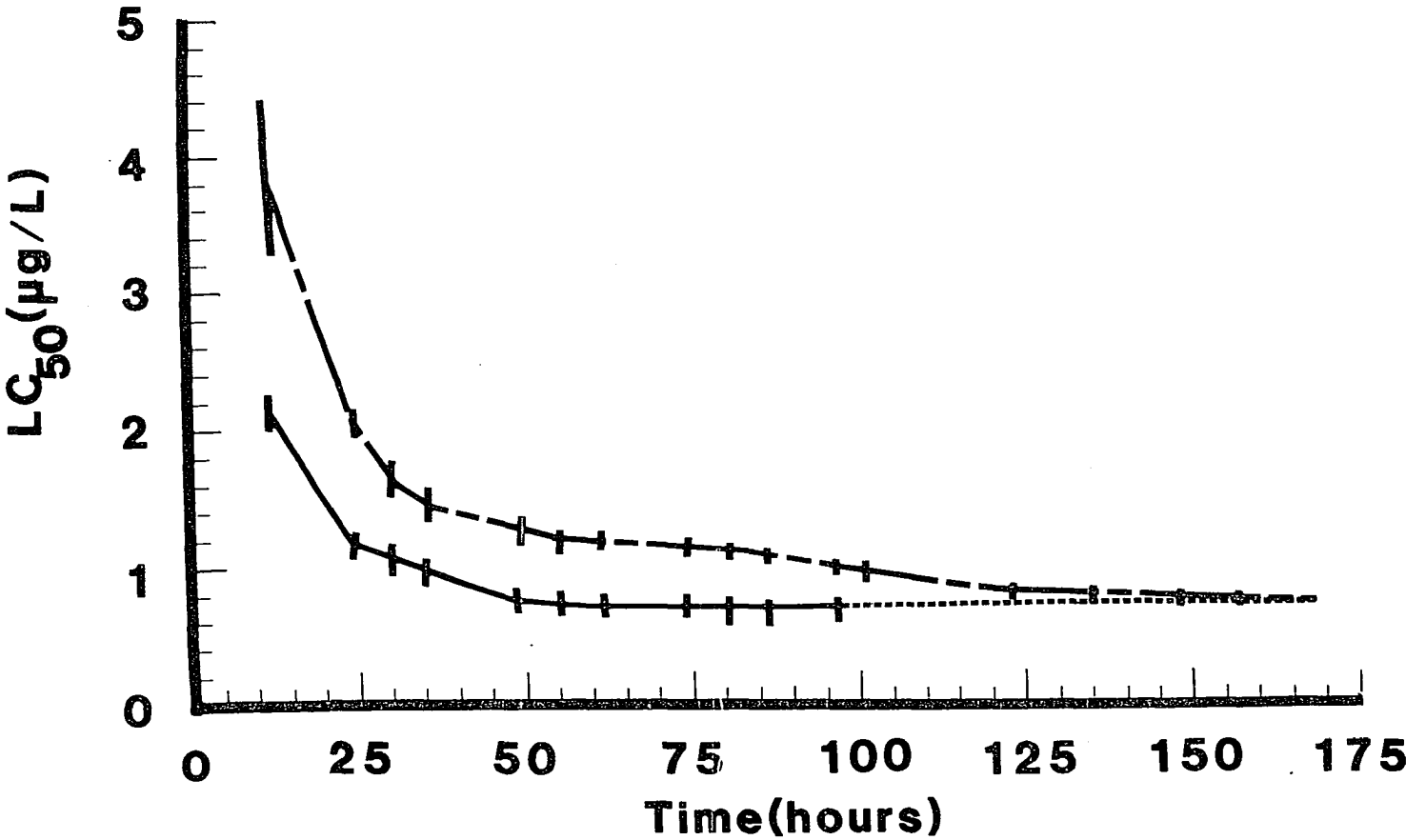


Fig. 1. Lethality of the technical-grade (solid line) and emulsifiable concentrate (dashed line) formulations of fenvalerate to fathead minnows over time

Bars represent 95% confidence intervals.

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

Formulation	LC_{50} (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.

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 exposures (decreasing residues with increasing fenvalerate water concentrations). These higher residue concentrations could be an aberration 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.

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 conc. (µg/L)[c]		Residue conc. (ng/g)	Time to mortality (h)	Net uptake rate (ng/g/h)	CF
Technical	ND[d]	(S)[e]	ND[d]	--------	--------	0 ± 0
	0.37 ± 0.13[f]	(S)	598 ± 124	--------	--------	1620 ± 335
	0.49 ± 0.12	(S)	911 ± 147	--------	--------	1860 ± 301
	0.75 ± 0.11	(S)[g]	1680	--------	--------	2240
	0.75 ± 0.11	(D)	1220 ± 122	45 ± 10	28 ± 7	1620 ± 162
	1.33 ± 0.10	(D)	1050 ± 79	25 ± 2	39 ± 6	791 ± 60
	3.51 ± 0.41	(D)	1010 ± 109	11 ± 1	93 ± 14	288 ± 31

[a]Reported on a whole body wet weight basis, not corrected for recovery.

[b]Uptake rate = $\frac{\text{Residue concentration}}{\text{Time to mortality}}$

[c]Concentrations based on percent active ingredient.

[d]ND, not detectable (<0.01 µg/L in water, <0.01 ng/g in tissue).

[e]Values obtained from samples derived from fish surviving fenvalerate exposure are denoted by (S), while those samples derived from fish which died are denoted by (D).

[f]Mean ± standard deviation (N = 4, except for technical 0.75(S) residue conc. where N = 1 and EC 1.49(D) residue conc. where N = 3).

[g]Data from these exposure concentrations were not used in statistical analyses, see text.

Table 2. Continued

Formulation	Mean water conc. (µg/L)[c]		Residue conc. (ng/g)	Time to mortality (h)	Net uptake rate (ng/g/h)	CF
30%	ND[d]	(S)	ND[d]	--------	--------	0 ± 0
Emulsifiable	0.58 ± 0.03	(S)	887 ± 76	--------	--------	1530 ± 130
Concentrate	0.92 ± 0.05	(D)	1300 ± 99	107 ± 5	12 ± 1	1410 ± 107
	1.49 ± 0.13	(D)	1170 ± 64	42 ± 7	28 ± 6	784 ± 43
	3.22 ± 0.14	(D)	1030 ± 89	18 ± 3	53 ± 9	321 ± 28
	8.11 ± 0.59	(D)[g]	1510 ± 145	11 ± 2	134 ± 15	187 ± 3

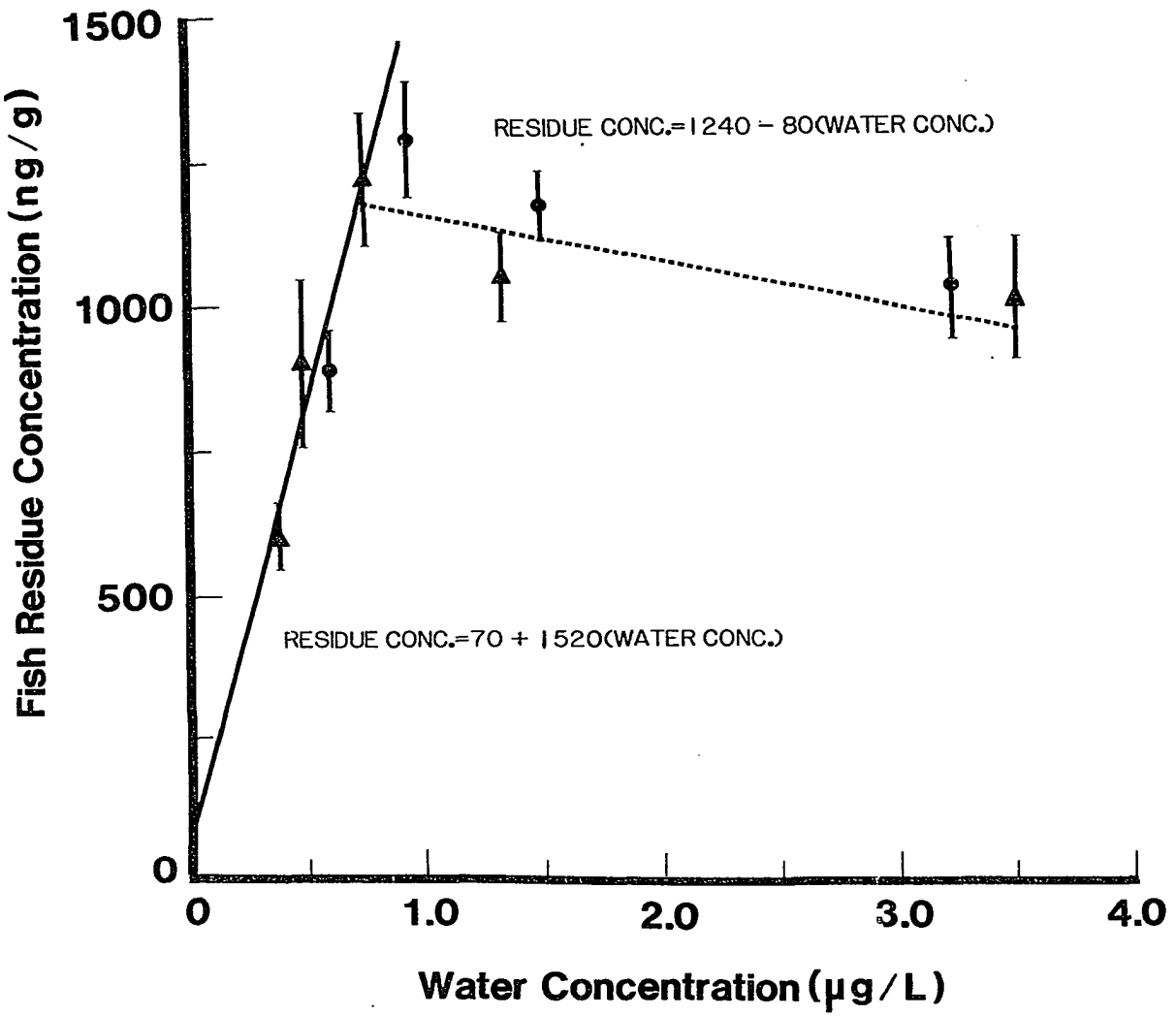
Analysis of covariance was performed on residue data for surviving fish and dead fish. Fenvalerate concentrations in surviving fish included those of the controls and fish exposed to fenvalerate exposures up to and including 0.75 and 0.92 µg/L from the technical-grade and EC tests, respectively. These 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 ($\pm_{20}$ = 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 describing the uptake of fenvalerate (Table 2). At comparable exposure concentrations, similar values were determined for each formulation, with

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.


1500
1000
500
0
Fish Residue Concentration (ng/g)
RESIDUE CONC.=1240 − 80(WATER CONC.)
RESIDUE CONC.=70 + 1520(WATER CONC.)
0
1.0
2.0
3.0
4.0
Water Concentration (µg/L)

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

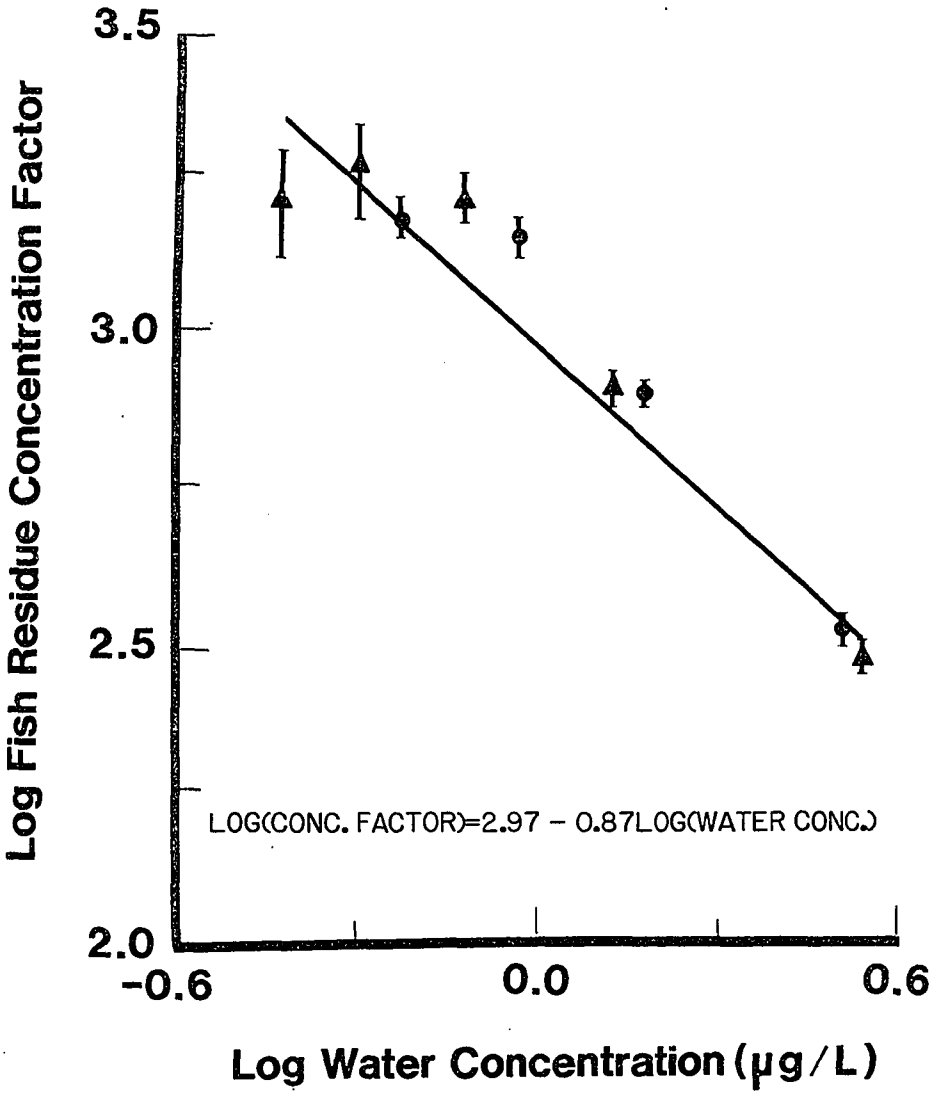


Fig. 3. Relationship between the log fish residue concentration factor and log fenvalerate water concentration for technical-grade (triangles) and emulsifiable concentrate (circles) formulations

Bars represent ± 1 SD. Concentration factors associated with the lowest three water concentrations were derived from data for fish that survived lethality testing.

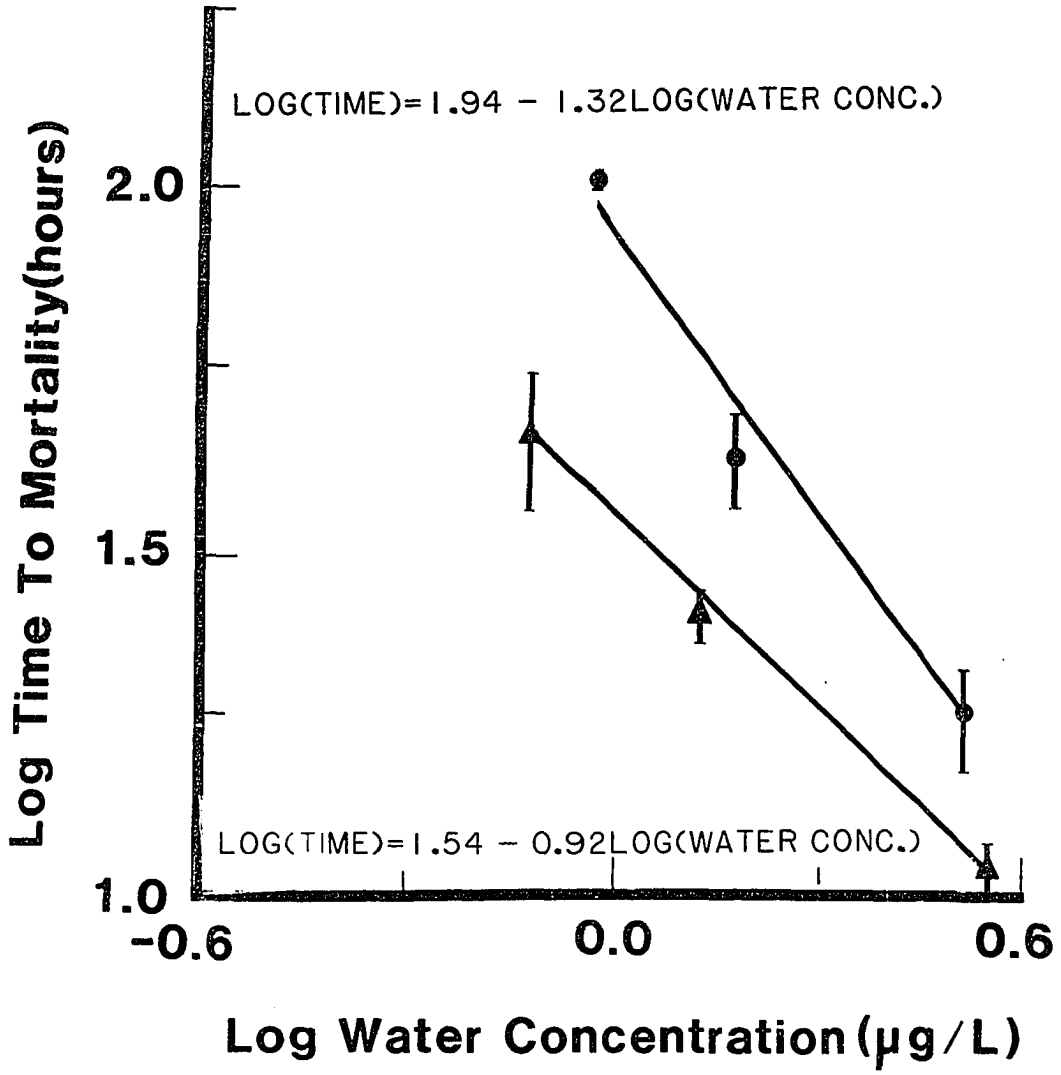


Fig. 4. Relationship between log time to mortality and log fenvalerate water concentration for fish exposed to technical-grade (triangles) or emulsifiable concentrate (circles) formulations

Bars represent ± 1 SD.

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.

DISCUSSION

The LC_{50} 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 LC_{50} 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 LC_{50}s of 2.15 to 2.35 µg/L. Flow-through 96-h LC_{50}s 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 LC_{50} 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 LC_{50} 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 LC_{50} of 76 µg/L (nominal) for rainbow trout, whereas McLeese et al. (17) reported a static 96-h LC_{50} of 1.2 µg/L for Atlantic salmon. In static tests with EC formulations of the insecticide, using rainbow trout, a 24-h LC_{50} of 21 µg/L (nominal) (2) and a 48-h LC_{50} 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 LC_{50}s 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 physiochemical interactions from combinations of aquatic pollutants and the resultant impact on biological systems.

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CHAPTER II. 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 fenvalerate (0.28 or 23 ng/L) or an emulsifiable concentrate formulation (16 ng/L) for 36 to 48 h. No significant effect of emulsifiers or fenvalerate concentration on uptake was 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 80-90 and 10-20% 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 exposure termination. 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 (7000 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 is not a contributing factor in fenvalerate's toxicity to fish. 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 LC_{50}s 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: 1) sensitivity at the site(s) of action, 2) highly efficient gill uptake, and 3) inefficient metabolism and elimination.

Efficient uptake of insecticides across the gills and into the blood-stream 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's, of 6.2 to 7.5) pyrethroids may be efficiently absorbed across the gills (7); however, specific research supporting this view is lacking.

Insufficient rates of pyrethroid-detoxification could also contribute to their lethality; 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 rate of hydrolysis and oxidation than that noted in mammals and birds, species less sensitive to pyrethroids (11). Greater fish toxicity with 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 adequately explained by differences in lipophilicity and uptake (5,6,12). The absence of toxicokinetic studies with α-cyano pyrethroids in fish makes an 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 fenvalerate's *in vivo* gill uptake, distribution, and elimination; the second included an examination of fenvalerate's *in vivo* metabolism. The respirometer-metabolism chambers, exposure system, and surgical procedures used to study the uptake and metabolism of fenvalerate were basically as described previously (13,14,15).

Toxicant Preparation and Exposure

[^{3}H]Fenvalerate (aromatic) was used in the study (Figure 1). Technical insecticide, obtained from the Shell Development Company, Modesto, CA, was tritiated via a catalytic exchange reaction (Amersham Corp., Arlington Hts., 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 >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/mmole, as determined by LSC and GLC (see (1) for GLC conditions; TLC systems are described in the Analysis of Biliary Metabolites section). [^{3}H]Fenvalerate with specific activities of 5.0 and 0.11 Ci/mmole (original material diluted with unlabeled insecticide) were used in the metabolism and uptake experiments, respectively. The material was dissolved in benzene:toluene (99:1) and stored at 4°C until used.

A fresh stock solution of [^{3}H]fenvalerate was prepared with distilled water in an 18-L stock bottle for each exposure period. Required aliquots

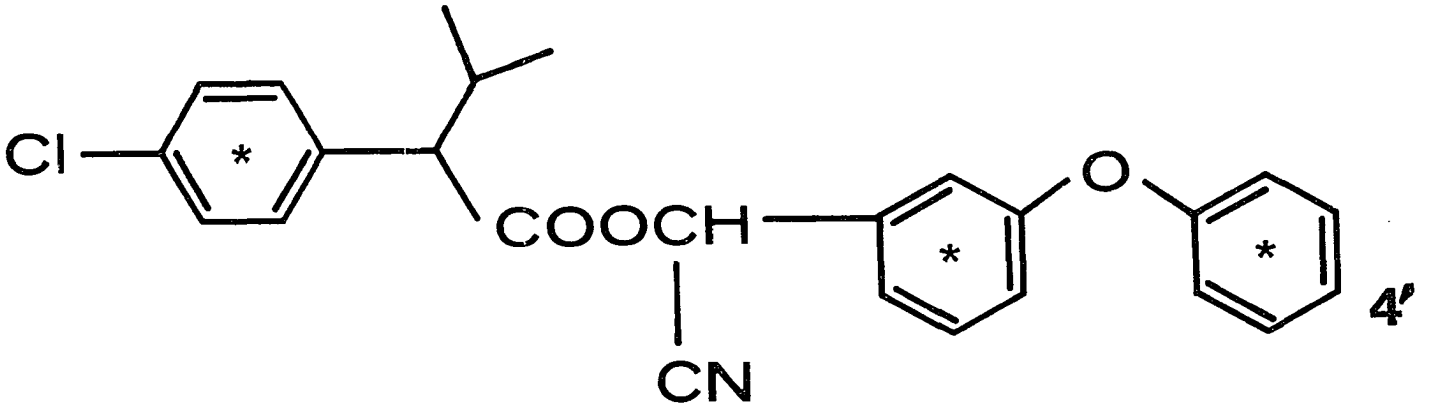

Fig. 1. Structure of fenvalerate showing ^{3}H-labeling positions (*) and site (4') of metabolic oxidation in rainbow trout

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 (the commercial EC, 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 1000 ml/min. The fenvalerate solutions flowed into two replicate metabolism chambers at a rate of 300 or 500 ml/min. Mean fenvalerate aqueous concentrations 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 - 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 Hts., IL), ranged from 10.5 to 11.0 (N=224). Mean pH (N=8) was 7.79 ± 0.06.

Fish Preparation

Four rainbow trout were used in the metabolism study (two females and two males), whereas in the uptake study, four were used with each

formulation (technical - three females and one male; EC - one female and three males). The trout used in this study were maintained at the USEPA Environmental Research Laboratory-Duluth, Duluth, MN, for several months before use and acclimated to a temperature of 11-12°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 fish 24 h before use.

Fish were exposed to fenvalerate in plexiglass respirometer-metabolism chambers (15). Fish were initially anesthetized with 100 mg/L of tricaine methanesulfonate (MS222, Finquel, Ayerst Laboratories, NY) and immobilized by spinal transection. Forty-eight hours later, fish were reanesthetized, and a latex rubber-oral membrane was sutured around the mouth to separate inspired and expired water. Fish were also fitted with urinary catheters (PE-60, polyethylene tubing) and fecal catheters. The fecal catheter consisted of a 10-cm Teflon tube with a small latex bag attached to the posterior end. After surgery, fish were placed in the metabolism chambers, and exposure of [^{3}H]fenvalerate began approximately 24 h later.

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, and elimination was monitored for either 48 h (technical) or 8 h (EC). During each exposure period, eight measurements of ventilation rate, ventilation

volume, DO uptake (see (15) for methods), and fenvalerate uptake were made. Gill uptake efficiency of oxygen and fenvalerate 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 preceding toxicant exposure (N=4 measurements per fish).

Urine (2 ml minimum sample) was collected at various times throughout the exposure and depuration periods. At the end of the depuration period, the fish were sacrificed by anesthetic overdose, and fecal material was collected via the catheter. Blood 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 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, gill, and muscle 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 of hexane was collected

in a scintillation vial and concentrated to 2 to 3 ml. Fifteen milliliters 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 ± 2% (N=8) recovery. During depuration periods in the metabolism study, expired water was re-extracted with ethyl acetate in an attempt to recover any radioactivity associated with more polar metabolites. Ten milliliters of MP cocktail (Beckman) was used to count aliquots of the ethyl acetate extract.

[^{3}H]Fenvalerate equivalents were determined in 3 to 4 subsamples from each tissue matrix collected from the fish. Urine samples (1 ml) were counted directly in 10 ml MP cocktail. Remaining matrices were analyzed following homogenization and solubilization. Whole blood (250 µl), packed cells (100 µl), plasma (100 µl), bile (25 µl), carcass (150-250 mg), and remaining tissues (150-250 mg) were digested in glass scintillation vials (3-18 h at 40-50°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 (1-3 h) to decolorize the samples. Digests were counted after the addition of 100-200 µl of glacial acetic acid and 15 ml of MP cocktail. Fecal samples were mixed by hand, and samples of the suspension (100-125 mg) were then oxidized in LSC vials with 200 µl of 60% perchloric acid and 400 µl of 30% H_2O_2 at 60°C for 3 h. After cooling, 15 ml of MP cocktail was added. Spiked samples indicated no loss of tritium after heating.

Radioactivity in water and tissue samples was quantified by 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 - 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 3X 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 (17) for methods). Additional aliquots were incubated with aryl sulfatase (Sigma). After incubation and acidification with HCl, the enzyme-bile extract solutions were extracted 3X 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 x 20 cm plates with a thickness of 0.25 mm (MCB Reagents, Gibbstown, NJ) and the following solvent systems: A, hexane:toluene:acetic acid (3:15:1), two developments; and 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, 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 by using two dimensional chromatography (system A followed by system B; adapted from (18)). Standards were visualized with ultraviolet light (254 nm). Radioactive sites on the plates were detected by radioautography (LKB Ultrofilm ^{3}H, LKB Instruments) and confirmed and quantified by scraping appropriate segments of the plates and measuring by LSC. Biliary metabolities were identified through cochromatography with unlabeled standards.

Statistical Analysis

T-tests were used to compare respiratory function, fenvalerate uptake, and fenvalerate distribution data. 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 pre-exposure, exposure, and depuration values, suggesting no sublethal effect of the insecticide. 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 fenvalerate gill-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 with the two formulations was noted. One fish in the EC group had a substantially higher ventilation volume (371 ml/min) and fenvalerate uptake efficiency (39.2%) than the remaining three fish. EC uptake data, excluding this aberrant fish, are also included in Table 2. Fenvalerate uptake efficiency in the technical group showed a slight decrease over time, whereas in the EC group, the opposite trend was noted. Regression analysis of fenvalerate uptake over time, however, indicated that the slopes within each formulation were not significantly different from 0. During the depuration periods, no measurable elimination of fenvalerate across the gills was noted.

The total [^{3}H]fenvalerate equivalents absorbed by each fish were calculated by multiplying the aqueous fenvalerate concentration by

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

	Pre-exposure	Exposure	Depuration
Ventilation Rate (No./min)			
UPTAKE STUDY			
Technical Formulation[a]	58 ± 10[b]	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]Fish were exposed to 23.2 ± 5.1 ng/L fenvalerate.

[b]Mean ± standard error for N=4 fish per experiment (N=12 for overall means). Functions were measured 4, 8, and 3-8 times per fish during pre-exposure, exposure, and depuration periods, respectively.

[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 = $\frac{\text{inspired } [O_2] - \text{expired } [O_2]}{\text{inspired } [O_2]} \times 100\%$

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

[^{3}]Fenvalerate equivalents in water (ng/L)	[^{3}H]Fenvalerate uptake efficiency[a]	Ventilation volume (L/h)	Exposure time (h)
	UPTAKE STUDY		
	Technical formulation		
23.2 ± 5.1[d]	0.236 ± 0.10	11.1 ± 2.4	47.8 ± 0.3
	Emulsifiable Concentrate Formulation		
15.6 ± 1.6	0.303 ± 0.064	13.7 ± 6.6	41.8 ± 6.6
(15.3 ± 1.9[e])	(0.273 ± 0.029)	(10.8 ± 4.0)	(43.7 ± 6.6)
	METABOLISM STUDY		
0.28 ± 0.09	0.321 ± 0.132	8.73 ± 1.31	46.5 ± 0.3

[a]Uptake efficiency = $\frac{\text{inspired [}^3\text{H]fenvalerate-expired [}^3\text{H]fenvalerate}}{\text{inspired [}^3\text{H]fenvalerate}}$.

[b]Water concentration x uptake efficiency x ventilation volume x exposure time; calculated for each fish separately.

[c][^{3}H]Fenvalerate equivalent concentration/exposure time.

[d]Mean ± standard error based on N=4 fish per experiment. Water concentrations, uptake efficiencies, and ventilation volumes were determined 8 times per fish during an exposure period.

[e]Ventilation volume and fenvalerate uptake for one fish in this experiment were substantially higher than those noted in the other fish. This second line of data excludes the aberrant fish; i.e., N=3.

Total [^{3}H]fenvalerate equivalents[b] (ng)	Fish weight (kg)	[^{3}H]Fenvalerate equivalents concentration in whole fish (ng/kg)	Dose[c] (ng/kg/h)
		UPTAKE STUDY	
		Technical Formulation	
2740 ± 910	0.777 ± 0.124	3580 ± 1190	74.9 ± 24.9
		Emulsifiable Concentrate Formulation	
2690 ± 1660	0.860 ± 0.098	3290 ± 2430	78.9 ± 58.2
(1860 ± 196)	(0.897 ± 0.078)	(2090 ± 384)	(48.1 ± 8.8)
		METABOLISM STUDY	
35.6 ± 13.9	0.894 ± 0.045	39.4 ± 17.1	0.85 ± 0.37

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 of [^{3}H]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 of 48.1 ng/kg/h was determined for the EC group, whereas a dose of 74.9 ng/kg/h was calculated for the technical group. The lower dose in the EC group is a function of the proportionately lower fenvalerate exposure concentration.

By quantifying the [^{3}H]fenvalerate equivalents in each fish and dividing by fish weight and exposure time, measured doses (Table 3) were determined to evaluate the accuracy of the calculated doses. Mean measured doses 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 were 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 is listed in Table 5. 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.

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

[^{3}H]Fenvalerate equivalents in remaining carcass[a] (ng)	[^{3}H]Fenvalerate equivalents in whole blood (ng)	[^{3}H]Fenvalerate equivalents in bile (ng)	[^{3}H]Fenvalerate equivalents in feces (ng)
	UPTAKE STUDY		
	Technical Formulation (23.2 ng/L)		
1994 ± 599[c]	ND[d]	213 ± 132	16.5 ± 11.3
	Emulsifiable Concentrate Formulation (15.6 ng/L)		
1260 ± 244	7.55 ± 0.47	142 ± 88	7.20 ± 0.90
	METABOLISM STUDY (0.28 ng/L)		
20.43 ± 2.96	0.12 ± 0.04	6.62 ± 1.40	0.10 ± 0.12

[a]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.

[b]Dose = Total [^{3}H]fenvalerate equivalents/fish weight/exposure period.

[c]Mean ± standard error based on N=4 fish per experiment.

[d]Not detectable. Limit of detection for blood in this study was 0.38 ng/ml.

[^{3}H]Fenvalerate equivalents in urine (ng)	Total [^{3}H]fenvalerate equivalents (ng)	[^{3}H]Fenvalerate equivalents concentration in whole fish (ng/kg)	Dose[b] ng/kg/h
	UPTAKE STUDY		
	Technical Formulation (23.2 ng/L)		
24.5 ± 18.7	2250 ± 630	2950 ± 878	61.7 ± 18.1
	Emulsifiable Concentrate Formulation (15.6 ng/L)		
20.9 ± 2.9	1437 ± 236	1682 ± 310	41.6 ± 13.1
	METABOLISM STUDY (0.28 ng/L)		
0.98 ± 0.51	27.9 ± 4.1	31.2 ± 4.9	0.67 ± 0.10

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

Tissue	Percentage of calculated dose[a]		
	UPTAKE STUDY		METABOLISM STUDY
	Technical Formulation (23.2 ng/L)	Emulsifiable Conc. Formulation (15.6 ng/L)	(0.28 ng/L)
Remaining carcass	74.1 ± 7.4[b]	72.3 ± 2.5[c]	66.6 ± 28.4[b]
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	83.5 ± 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 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. See Table 3 footnotes.

Table 5. Distribution of [^{3}H]fenvalerate equivalents in rainbow trout based on a measured dose

Tissue	Percentage of measured dose[a]		
	UPTAKE STUDY		METABOLISM STUDY
	Technical Formulation (23.2 ng/L)	Emulsifiable Conc. Formulation (15.6 ng/L)	(0.28 ng/L)
Remaining carcass	88.8 ± 6.7[b]	87.5 ± 6.5	72.8 ± 3.0
Blood	ND[c]	0.5 ± 0.1	0.5 ± 0.2
Bile	9.3 ± 5.7	10.0 ± 6.4	23.6 ± 2.2
Feces	0.8 ± 0.6	0.5 ± 0.2	0.4 ± 0.5
Urine	1.1 ± 0.9	1.5 ± 0.3	3.5 ± 2.1
Total	100	100	100

[a]See Table 3 for measured doses.

[b]Mean ± standard deviation based on N=4 fish.

[c]Not detectable. See Table 3 footnotes.

With both formulations, significant elimination and blood transport of [^{3}H]fenvalerate equivalents was completed within 8 to 12 h after exposure termination. 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 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 fenvalerate gill-uptake efficiency of 32.1% (Table 2) in this study was not significantly different from that determined in the uptake study. The mean calculated dose for the four fish was 0.85 ng/kg/h (Table 2), and the mean measured dose 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 (Tables 4 and 5). 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 with the metabolism study (70%) than with the uptake study (90%). The percentage of fenvalerate equivalents in the urine fraction and the time course of excretion was similar to that 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 6). Bile concentrations (about 7000 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 tissue, slightly higher concentrations were found in the gills, liver, kidneys, and packed blood cells (about 45 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.

Table 6. Concentration of [^{3}H]fenvalerate equivalents in rainbow trout tissues after a 48-h aqueous exposure (0.28 ng/L) and 48 h of depuration

Tissue	[^{3}H]Fenvalerate equivalents (pg/g)[a]
Bile	7060 ± 4910
Blood (whole)	22 ± 7
Brain	21 ± 11
Carcass (ramaining)	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 where N=2. Range for heart and ovaries were 13-21 and 25-30 pg/g, respectively.

[b]Not detectable. Limit of detection for testes was 10 pg/g.

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 fenvalerate gill-uptake efficiencies derived from the uptake study and metabolism study 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 PCB 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 (<1 to 7.5). Results with fenvalerate, at a calculated log p of 7.2 (20), agree closely with that of mirex (log p of 7.5; 20% uptake efficiency) and indicate that, at log p's between 6 to 7, gill-uptake efficiency drops from about 60 to 20-30%.

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 these levels, significant interactions with emulsifying agents may result. In the present study, fenvalerate concentrations were well below solubility limits.

Distribution of [^{3}H]fenvalerate equivalents indicate 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 with permethrin after aqueous and i.p. exposures to rainbow trout (10), with some indication that fenvalerate and its metabolites may be less readily eliminated. In contrast, warm-blooded vertebrates are very efficient in eliminating fenvalerate and its metabolites. 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 routes of exposure; presumably, an i.v. exposure in mammals would more closely mimic gill-uptake exposures 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 an oral exposure (23).

Analysis of specific tissues for [^{3}H]fenvalerate equivalents after a 48-h depuration period indicated 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 the present study. Permethrin was also found to concentrate in the fat of rainbow trout (10). Accumulation in fat is likely 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 the plasma. Typically, insecticides are transported in the plasma fraction of the blood (25). The results noted in this study further underscore the rapid distribution of fenvalerate into lipid compartments within trout.

Analysis of biliary metabolites indicated oxidation at the 4' position, followed by glucuronidation, to be 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 metabolism and excretion of fenvalerate by rainbow trout is 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 2, 4, 5, 6, 12 for comparative toxicity data). The extent and nature of fenvalerate metabolism in trout is markedly different from that 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 a variety of oxidative (including

4'-HO-fenvalerate) and hydrolytic products (18,24). Similar levels of metabolism have been found with bobwhite quail orally administered fenvalerate (21).

Results from 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 is not a contributing factor in fenvalerate's extreme toxicity. Even though gill uptake is inefficient, relative to many other xenobiotics, direct uptake of fenvalerate into the bloodstream is an important consideration. Route of administration in mammals does influence lethality; fenvalerate is more toxic to rats after an i.v. exposure (50-100 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 fenvalerate's piscicidal activity, presumably by resulting in 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 permethrin's toxicity 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 fenvalerate

exposure may also be important contributing factors. It is hoped that research in progress will help to elucidate the toxic mode of action of fenvalerate in rainbow trout.

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SUMMARY

Several aspects regarding the aquatic toxicology of fenvalerate, a synthetic pyrethroid insecticide are addressed in this study.

An initial investigation involved the influence of emulsifiers on the lethality and uptake of the insecticide in fathead minnows. Results from this study indicated that technical fenvalerate was initially more toxic (96-h LC_{50} of 0.69 µg/L) than a 30% active ingredient EC formulation (96-h LC_{50} of 0.99 µg/L); however, the incipient lethalities were similar (168-h LC_{50} for the EC of 0.75 µg/L). Fenvalerate concentration factors and residue levels in the fish were not significantly different between formulations. The time required to accumulate lethal residues, however, was longer with the EC and contributed to a significantly slower uptake rate. In turn, this slower uptake was seemingly responsible for the initial difference in toxicity. The lower EC uptake rate suggests that an interaction between fenvalerate and the emulsifiers reduces the bioavailability of the insecticide. Results of previous studies (26,27), indicated that commercial emulsifiers enhanced the lethality of pyrethroids to fish. The discrepancy between studies probably reflects differences in methodologies (static tests with nominal aqueous fenvalerate concentrations as opposed to flow-through tests with measured concentrations). The findings of this study indicate no additional acute environmental hazard should be incurred with the use of formulated pyrethroid insecticides. In addition, these results suggest that the use of carrier solvents in aquatic toxicity evaluations may have little effect on incipient LC_{50}s, but may effect the rate at which toxic responses are manifested.

The second aspect of the project addressed the in vivo gill-uptake and toxicokinetics of [^{3}H]fenvalerate in rainbow trout. The overall mean gill-uptake efficiency was determined to be 28.6 ± 4.4%. Gill-uptake was independent of fenvalerate aqueous concentration. Fenvalerate's gill-uptake efficiency is consistent with a recently developed model (62) and suggests that at log p's > 6 gill uptake of xenobiotics becomes relatively inefficient. In contrast to the findings of the first study reported in this dissertation, no effect of the commercial emulsifiers on uptake was observed. Significant interactions between the insecticide and emulsifiers may occur only near the water solubility limit of fenvalerate. Distribution of [^{3}H]fenvalerate equivalents in trout indicated that this species is relatively inefficient in eliminating fenvalerate and its metabolites (80 to 90% of the dose remained in the fish). Bile contained the highest concentration of equivalents, followed by fat. High bile levels are consistent with fenvalerate's major route of excretion, while levels in the fat are likely a function of fenvalerate's high lipophilicity. Analysis of biliary metabolites indicated oxidation at the 4' position, followed by glucuronidation, to be the only significant degradation steps in rainbow trout. The data from this study suggest that efficient gill-uptake is not a contributing factor in fenvalerate's extreme toxicity to fish, although direct uptake into the blood stream is an important consideration. Rather, a low rate of biotransformation and excretion may play a significant role in the susceptibility of fish to the synthetic pyrethroid insecticides.

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APPENDIX. SUPPLEMENTAL DATA: PHYSIOLOGICAL RESPONSE OF RAINBOW TROUT TO LETHAL AQUEOUS CONCENTRATIONS OF FENVALERATE

The following tables present supplemental data regarding the *in vivo* response of rainbow trout to lethal aqueous levels of fenvalerate. Four trout were used in this investigation and prepared as described in Chapter II, except each fish was also fitted with a dorsal aorta cannula and heart electrodes. In addition to the physiological parameters described in Chapter II, gill ammonia excretion, gill-water pH, heart rate, blood chemistry, and urine chemistry were also monitored during the exposures. Finally, plasma ion levels and plasma osmolality, associated with mortality, were determined, as well as, fenvalerate concentrations in brain, liver, and carcass.

Table A.1. Characteristics of rainbow trout and exposure parameters

Fish No.	Sex	Weight (g)	Length of Exposure (h)[a]	Aqueous Fenvalerate Concentration (ng/L)[b]
112	female	622	11.0	423 ± 15[c]
113	male	919	12.8	382 ± 43
114	male	535	8.3	365 ± 48
115	male	759	10.0	477 ± 80
Mean ± SD		709 ± 168	10.5 ± 1.9	412 ± 50

[a]Exposure was terminated at time of death.

[b]Fenvalerate was added to the water in a DMF solution, with a final nominal aqueous DMF concentration in the metabolism chambers of 200 ppm. Water was sampled as described in Chapter II and analyzed by GLC as outlined in Chapter I. These concentrations are derived from inspired water.

[c]Mean ± SD; based on four samples taken during the course of an exposure.

Table A.2. Physiology summary for fish 112

Exposure Time (h)	Ventilation Rate (No./min)	Ventilation Volume (ml/min)	Heart Rate (No./min)	Oxygen Uptake Efficiency (%)[a]	Ammonia-Nitrogen (mg/L)		Water pH	
					Inspired Water	Expired Water	Inspired Water	Expired Water
-4.50	78	100	42	54	----[b]	----[b]	----[b]	----[b]
-4.20	78	112	42	53	----[b]	----[b]	8.02	7.91
-3.80	78	125	44	53	0.056	0.743	7.96	7.80
-2.70	76	90	42	55	0.069	1.000	7.96	7.71
0.83	76	110	44	56	0.070	0.846	8.08	7.53
2.00	72	100	42	56	0.055	1.012	7.98	7.49
4.33	78	200	48	27	0.081	0.423	8.09	7.64
6.00	100	262	52	23	----	----	7.92	7.62
7.80	70	250	46	49	0.052	0.154	7.96	7.37
8.80	90	125	32	59	0.044	0.418	7.03	7.30
9.58	53	62	31	68	----[b]	----[b]	----[b]	----[b]
10.67	29	----[b]	7	----[b]	----[b]	----[b]	----[b]	----[b]

[a] Oxygen uptake efficiency = $\frac{\text{Inspired } [O_2] - \text{Expired } [O_2]}{\text{Inspired } [O_2]} \times 100\%$

[b] Not measured.

Table A.3. Physiology summary for fish 113

Exposure Time (h)	Ventilation Rate (No./min)	Ventilation Volume (ml/min)	Heart Rate (No./min)	Oxygen Uptake Efficiency (%)[a]	Ammonia-Nitrogen (mg/L)		Water pH	
					Inspired Water	Expired Water	Inspired Water	Expired Water
-4.50	69	225	36	47	----[b]	----[b]	----[b]	----[b]
-4.20	70	262	40	45	----[b]	----[b]	8.02	----[b]
-3.80	--[b]	213	44	47	0.066	0.626	8.00	7.70
-2.70	70	215	44	45	0.066	0.906	7.96	7.61
0.83	60	200	36	46	0.065	0.812	7.92	7.50
2.00	62	212	36	46	0.061	0.599	8.05	7.45
4.33	64	225	38	43	0.075	1.073	7.98	7.47
6.00	94	238	46	50	----[b]	----[b]	7.85	7.42
7.80	93	288	40	52	0.0701	0.465	7.85	7.52
8.80	42	250	25	48	0.094	0.326	7.82	7.40
9.58	71	250	24	46	----[b]	----[b]	----[b]	----[b]
12.08	49	60	15	32	----[b]	----[b]	----[b]	----[b]

[a] Oxygen uptake efficiency = $\frac{\text{Inspired } [O_2] - \text{Expired } [O_2]}{\text{Inspired } [O_2]} \times 100\%$

[b] Not measured.

Table A.4. Physiology summary for fish 114

Exposure Time (h)	Ventilation Rate (No./min)	Ventilation Volume (ml/min)	Heart Rate (No./min)	Oxygen Uptake Efficiency (%)[a]	Ammonia-Nitrogen (mg/L)		Water pH	
					Inspired Water	Expired Water	Inspired Water	Expired Water
-2.83	68	75	34	59.8	----[b]	----[b]	7.81	7.51
-2.25	70	113	36	59.8	----[b]	----[b]	7.92	7.43
-1.25	78	87	32	57.7	0.0044	1.024	7.89	7.42
0.83	68	87	40	65.5	----[b]	1.023	7.89	7.47
1.67	68	100	44	59.4	0.082	1.010	7.80	7.39
3.00	80	100	44	49.1	----[b]	----[b]	----[b]	----[b]
3.67	100	113	48	44.5	0.1120	1.090	7.94	7.47
5.42	33	200	26	----[b]	0.1140	0.782	7.97	7.41
7.42	40	50	22	60.9	0.580	0.520	7.88	7.45

[a]Oxygen uptake efficiency = $\frac{\text{Inspired } [O_2] - \text{Expired } [O_2]}{\text{Inspired } [O_2]} \times 100\%$.

[b]Not measured.

Table A.5. Physiology summary for fish 115

Exposure Time (h)	Ventilation Rate (No./min)	Ventilation Volume (ml/min)	Heart Rate (No./min)	Oxygen Uptake Efficiency (%)[a]	Ammonia-Nitrogen (mg/L)		Water pH	
					Inspired Water	Expired Water	Inspired Water	Expired Water
-2.83	----[b]	150	----[b]	56.5	----[b]	----[b]	7.90	7.53
-2.25	68	150	36	57.9	----[b]	----[b]	7.89	7.40
-1.25	68	250	34	69.1	ND[c]	1.810	8.09	7.43
0.83	72	150	52	59.3	----[b]	0.978	7.87	7.36
1.67	64	125	44	61.6	0.085	1.392	8.02	7.41
3.00	88	150	50	57.3	----[b]	----[b]	----[b]	----[b]
3.67	102	150	38	60.0	0.086	0.691	8.20	7.52
5.42	46	87	34	51.4	0.151	0.175	7.82	7.27
7.42	42	200	26	49.5	0.058	0.090	8.07	7.43
8.33	42	116	27	45.5	----[b]	----[b]	----[b]	----[b]
9.17	81	50	24	43.6	----[b]	----[b]	----[b]	----[b]

[a] Oxygen uptake efficiency = $\frac{\text{Inspired } [O_2] - \text{Expired } [O_2]}{\text{Inspired } [O_2]} \times 100\%$.

[b] Not measured.

[c] Not detectable.

Table A.6. Blood parameters for fish 112

Exposure Time (h)	Total O_2 (g/100ml)	pO_2 (mmHg)	Total CO_2 (mmole/L)	pCO_2 (mmHg)	Blood pH	Hematocrit (%)	Hemoglobin (g/100ml)
-0.67	4.83	18.49	10.79	1.06	8.09	20.75	6.74
0.83	6.43	24.58	11.97	1.18	8.19	19.25	6.36
2.58	5.31	20.32	10.79	1.06	8.06	18.0	7.31
8.08	6.54	25.03	9.21	0.89	8.10	25.5	8.46
10.00	0.94	3.61	7.24	0.69	7.57	31.5	8.40

Table A.7. Blood parameters for fish 113

Exposure Time (h)	Total O_2 (g/100ml)	pO_2 (mmHg)	Total CO_2 (mmole/L)	pCO_2 (mmHg)	Blood pH	Hematocrit (%)	Hemoglobin (g/100ml)
-0.17	3.79	14.51	12.37	1.22	8.04	21.0	7.01
1.67	8.08	30.89	9.60	0.93	8.10	23.5	7.05
3.00	8.07	30.85	10.39	1.02	----[a]	22.5	7.15
10.58	3.21	12.27	5.66	0.53	7.90	30.0	6.19

[a]Not measured.

Table A.8. Blood parameters for fish 114

Exposure Time (h)	Total O_2 (g/100ml)	pO_2 (mmHg)	Total CO_2 (mmole/L)	pCO_2 (mmHg)	Blood pH	Hematocrit (%)	Hemoglobin (g/100ml)
-0.30	10.03	38.37	7.48	0.56	7.86	24.5	9.34
2.17	7.53	28.79	5.83	0.44	7.58	22.8	8.40
4.33	10.50	40.16	5.83	0.44	7.80	35.0	10.43
6.25	3.61	13.79	4.19	0.32	7.47	40.5	9.75
7.58	1.95	7.47	3.64	0.28	7.45	43.0	8.20

Table A.9. Blood parameters for fish 115

Exposure Time (h)	Total O_2 (g/100ml)	pO_2 (mmHg)	Total CO_2 (mmole/L)	pCO_2 (mmHg)	Blood pH	Hematocrit (%)	Hemoglobin (g/100ml)
-0.17	4.06	15.53	5.83	0.44	7.95	23.5	7.60
2.50	3.43	13.11	5.83	0.44	7.87	18.5	7.69
4.50	7.56	28.91	5.83	0.44	7.90	23.5	8.62

Table A.10. Urine parameters for fish 112

Exposure Time (h)	Urine Volume (ml)	Urine pH	Urine Osmolality (mosm/L)	Urine Na^+ (mmole/L)	Urine K^+ (mmole/L)
-4.25	1.8	------[a]	18.5	8.66	1.79
-3.33	2.0	------[a]	19.0	8.51	1.71
-2.83	5.2	6.69	19.0	8.03	1.64
-1.00	4.2	------[a]	19.5	8.59	1.74
0.00	4.0	6.86	17.5	8.56	1.60
1.50	2.8	------[a]	19.5	8.43	1.50
2.50	6.6	7.78	18.5	8.60	1.55
4.33	7.8	------[a]	20.5	9.79	1.62
6.58	3.4	------[a]	18.5	8.68	1.57
7.83	5.4	6.75	23.5	14.50	3.40

[a]Not measured.

Table A.11. Urine parameters for fish 113

Exposure Time (h)	Urine Volume (ml)	Urine pH	Urine Osmolality (mosm/L)	Urine Na^+ (mmole/L)	Urine K^+ (mmole/L)
-4.25	1.4	6.98	92.5	35.79	3.13
-0.50	0.2	-----[a]	49.0	10.40	2.54
0.00	6.0	7.365	61.0	19.87	3.45
1.50	4.8	6.90	64.0	23.95	3.44
3.25	3.2	-----[a]	68.0	21.75	3.22
4.75	6.2	-----[a]	69.5	21.13	3.61
6.58	2.6	6.78	63.0	23.17	3.41
8.17	10.4	-----[a]	80.0	27.49	4.09
9.33	1.4	6.93	82.0	-----[a]	3.31

[a]Not measured.

Table A.12. Urine parameters for fish 114

Exposure Time (h)	Urine Volume(ml)	Urine pH	Urine Osmolality (mosm/L)	Urine Na^+ (mmole/L)	Urine K^+ (mmole/L)
-2.92	4.0	-----[a]	22.5	3.47	6.84
-1.92	2.6	-----[a]	22.0	3.99	6.98
-1.25	5.4	7.58	23.5	5.50	6.88
0.00	4.6	-----[a]	22.0	4.51	7.06
1.00	3.6	7.68	22.5	4.19	6.96
2.25	5.4	-----[a]	22.0	3.51	6.92
2.42	2.6	6.72	22.5	2.16	6.24
4.58	2.8	-----[a]	25.0	1.72	6.39

[a]Not measured.

Table A.13. Urine parameters for fish 115

Exposure Time (h)	Urine Volume (ml)	Urine pH	Urine Osmolality (mosm/L)	Urine Na^+ (mmole/L)	Urine K^+ (mmole/L)
-2.92	3.6	-----[a]	20.5	0.72	2.54
-1.92	1.8	-----[a]	23.5	-----[a]	2.24
-1.25	4.0	7.07	20.5	0.83	2.44
0.00	3.4	-----[a]	16.5	0.91	2.50
1.00	2.8	7.64	20.5	0.84	2.23
2.25	4.6	-----[a]	18.5	0.99	2.24
2.42	2.8	6.67	21.0	1.90	2.95
4.58	6.6	6.52	44.5	2.12	4.13
6.67	3.0	-----[a]	137.0	52.20	4.42

[a]Not measured.

Table A.14. Plasma osmolality and ion concentrations associated with mortality in rainbow trout

Fish No.	Osmolality (mOsm/kg)	Cl^- (meq/L)	Na^+ (mmole/L)	K^+ (mmole/L)	Ca^{2+} (mmole/L)	Mg^{2+} (mmole/L)
Control[a]	283 ± 10	118 ± 8	130 ± 7	2.44 ± 0.75	1.85 ± 0.06	0.53 ± 0.02
112	280	115	135	0.95	1.29	0.45
113	247	95	122	1.89	1.96	0.99
114	286	107	132	2.46	1.87	0.92
115	298	120	140	2.46	1.92	0.79
Mean ± SD[b]	278 ± 22	109 ± 11	132 ± 8	1.94 ± 0.71	1.76 ± 0.32	0.79 ± 0.24

[a]Control values from four additional rainbow trout (James M. McKim, U.S. EPA, Environmental Research Laboratory-Duluth, Duluth, MN, 1985; unpublished data).

[b]Based on Fish 112-115.

Table A.15. Fenvalerate residues associated with mortality in rainbow trout

Tissue	Residue Levels (ng/g) Fish No. 112	113	114	115	Mean ± SD
Brain	171	123	234	124	163 ± 52
Liver	3557	3141	4438	3355	3623 ± 569
Remaining carcass	245	191	302	284	256 ± 49